ORIGINAL ARTICLE

Hepatopathy in Victorian dogs consuming pet meat contaminated with indospicine

S. E. Salmon,[a*] S. L. Hall,[a] D. E. Phillips,[a] L. R. Dries,[a] J. Sarandopoulos,[a] A. Coghill[b] and M. C. Hawes[c]

Background Indospicine is an arginine analogue and a natural toxin occurring only in *Indigofera* plant species, including Australian native species. It accumulates in the tissues of grazing animals, persisting for several months after ingestion. Dogs are particularly sensitive to indospicine toxicity and can suffer fatal liver disease after eating indospicine-contaminated pet meat.

Method A disease outbreak investigation was launched following notification to Agriculture Victoria of a cluster of 18 dogs displaying acute, severe, hepatopathy in the East Gippsland Shire in June 2021.

Results Between June and September 2021, 24 pet dogs died, and 40 others experienced liver disease after eating commercially prepared pet meat found to contain indospicine. The investigation identified the toxin in serum and liver samples from affected dogs and at high levels in some samples of pet meat eaten by the dogs. Twenty-six horses that were moved from the Northern Territory and processed at a Pet Meat Processing facility (knackery) in eastern Victoria over a period of 14 days in late May–early June 2021 were identified as the likely source of the indospicine toxin in the pet meat. Pet meat produced by the knackery and on-sold by several retailers was determined to be the cause of the illness and death in the dogs.

Conclusion This is the first report of severe and frequently fatal hepatopathy in dogs in Victoria relating to consumption of pet meat contaminated with indospicine.

Keywords dogs; hepatopathy; indospicine; pet meat; toxicity

Abbreviations ALP, alkaline phosphatase; ALT, alanine aminotransferase; GGT, gamma-glutamyl transferase

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Indospicine (L-6-amidino-2-amino-hexanoic acid) is a non-proteinogenic, hepatotoxic, arginine analogue that is found naturally in some *Indigofera* plant species in Australia.[1] Grazing animals that consume *Indigofera* plants containing indospicine accumulate the toxin in their tissues including muscle[1] (and residues have been demonstrated to persist for months).[2] Ingestion of indospicine-contaminated horse meat[3] or camel meat[4] has been reported as the cause of severe hepatopathy in dogs, which as a species are particularly sensitive to indospicine. The risk of canine poisoning is well understood in northern Australia, and the Northern Territory *Meat Industry Act 1996*[5] states that a person shall not slaughter a horse, donkey, mule or hinny for pet food if it exhibits signs of being affected by Birdsville horse disease (a toxic condition of horses caused by eating Birdsville indigo [*Indigofera linnaei*][6]) or if it has been in an area in which Birdsville horse disease occurs.

Indospicine toxicity has not previously been reported in dogs in Victoria, but on 29 June 2021, veterinarians in two clinics in the Bairnsdale area of the East Gippsland Shire treated 10 dogs with acute onset, severe symptoms including inappetence, vomiting, lethargy and jaundice. In-house clinical chemistry testing of affected dogs revealed biochemical changes consistent with a severe hepatopathy. As the number of cases escalated, the veterinarians sought diagnostic assistance from Agriculture Victoria and flagged a possible, but at that stage unsubstantiated, association between the illness and a common source of pet meat fed to affected animals.

We report on the investigation and the epidemiological features of the incident that lasted 2 months and resulted in the deaths of 24 dogs, liver disease in at least a further 40, and was geographically spread across south-eastern Victoria and around Melbourne.

Materials and methods

Following the initial notification of the cluster of cases, Agriculture Victoria supported private veterinarians to undertake a significant disease investigation[7] facilitating testing of clinical samples (serum and liver) from affected dogs and aliquots of pet food provided by their owners. Because of the apparent (but circumstantial) link between the illness in the dogs and pet meat originating from a local knackery, PrimeSafe, the Statutory Authority responsible for pet food regulation in Victoria, was advised and subsequently involved in the investigative process.

The aims of the investigation were to describe the incident as completely as possible, assess the involvement of notifiable, zoonotic or exotic disease in the event, to support pet owners and their veterinarians to access expert advice on treating affected dogs, and ideally to accumulate sufficient information and high-quality samples to enable the cause of the hepatopathy to be identified, thereby preventing further cases.

*Corresponding author.
[a]Chief Veterinary Officer's Unit, Agriculture Victoria, Attwood, Victoria, Australia; sally.salmon@agriculture.vic.gov.au
[b]PrimeSafe, South Melbourne, Victoria, Australia
[c]Department of Jobs, Precincts and Regions, Agriculture Victoria Research, Bundoora, Victoria, Australia

Case definition and accumulation

An initial case definition of "clinical signs (including inappetence, lethargy and jaundice) or biochemical changes associated with liver dysfunction, in an otherwise previously healthy animal" was used. Reports of liver dysfunction amongst domestic animals (specifically dogs and cats but potentially also ferrets) were to be included in the initial data set. Comprehensive data were obtained from owners and vets on possible cases. The case definition was later refined to include only dogs when it became clear that no other species was affected.

Identification of cases from across Victoria was encouraged through the distribution to veterinarians, of an Agriculture Victoria "Biosecurity Alert" describing the event and the presenting signs. The Alert was provided through multiple channels including the email subscription list of an existing Agriculture Victoria newsletter aimed at private veterinarians. Cases were also identified by members of the public responding to information posted through traditional and social media releases. When the investigations team was contacted directly by owners of affected pets, the investigation team liaised with the owner's private veterinarian to review the pet's presentation and assess whether it met the case definition and could be included in the data set.

With appropriate consents, Agriculture Victoria staff collected detailed case histories on the affected animals and any unaffected animals in the household, from owners. Cases were classified as *substantiated* when their details and history were confirmed through personal contact with owners and/or veterinary practitioners. If cases could not be followed up further for any reason, they were classified as *anecdotal*.

Data analysis

Case data were accumulated in the Agriculture Victoria bespoke emergency response case management database ("MAX") with a campaign site specifically configured for this event, and data were visualised and analysed using Microsoft Power BI Desktop (64-bit, 2021), Microsoft® Excel® for Microsoft 365 and Tableau 10.2® 2003–2022 Tableau Software, LLC.

Differential diagnoses

A broad range of infectious and toxic causes was initially considered as potential differential diagnoses for the observed liver dysfunction observed (Table 1). Due to the geographic and temporal clustering of cases, from the outset, a common point source exposure was considered most likely. The list of possible causes was narrowed to exclude aetiologies with low likelihood based on expert opinion. Advice that the histopathological lesions observed in liver samples from affected dogs indicated a severe, sub-acute toxic hepatopathy further focused investigations on a toxic agent as the most likely cause, with aflatoxins, amatoxin and indospicine considered as potential feed contaminants. Potential sources of toxin were considered, including pet food, farm, industrial and household chemicals, medicines, poisons and baits, toxin-producing microbes and plant toxins (through primary ingestion of plant material by dogs, or secondary ingestion from contaminated meat sources).[8] The possibility that this incident may have been caused by an emerging infectious agent or previously undescribed hepato-toxic mechanism was also considered.

Laboratory analysis

Where possible, samples collected from affected dogs included serum and fresh/fixed liver (where dogs died or were euthanased due to hepatic failure). Haematological and biochemical tests were performed by private veterinarians either using in-house analysers or by private referral diagnostic laboratories. Histopathology of fixed liver tissue was examined at Veterinary Diagnostic Services – AgriBio – and fresh samples were divided with some retained at AgriBio, and the remainder of the sample referred to external laboratories for specific toxicology and meat speciation testing, where indicated. Where possible, samples of stored fresh or frozen pet meat were also collected from households of affected dogs, from retailers and wholesale suppliers that received meat from the eastern Gippsland knackery and these were also retained.

Samples of serum and liver from affected dogs were referred to the Queensland Alliance for Agriculture and Food Innovation (QAAFI), University of Queensland, for indospicine testing. Pet food samples were also referred to Agrifood Technology, Werribee for aflatoxin testing. No laboratory was identified in Australia that could conduct testing for amatoxin, so no analysis for this toxin could be undertaken. Meat speciation testing was conducted at Intertek Testing Services (Australia) in South Australia.

Case–control study

A case–control study[9] to identify exposures that may have been associated with cases of hepatopathy in dogs, and to facilitate identification of any point source of toxin was undertaken in parallel with diagnostic and toxicological testing.

PrimeSafe investigation

Under the *Meat Industry Act 1993, PrimeSafe* is the Statutory Authority responsible for regulating meat, poultry, seafood and pet food in Victoria. Pet meat processing facilities are licensed and audited by *PrimeSafe*, so when the link between the illness in dogs and consumption of knackery derived product was observed, *PrimeSafe* inspectors visited the premises to ensure that pet meat was being processed in compliance with the quality assurance programme in place, the Standard for the Hygienic Production of Pet Meat[10] and *PrimeSafe* Licence requirements. From the outset, inspectors sought information in relation to the livestock and game (type and source) and any other additives, preservatives or dyes, used in the production of their product. Once indospicine had been identified as a likely cause of the lesions seen in the dogs and camel meat was ruled out as never having been processed or received by the knackery, investigation at the knackery focused on the origins of horse meat that was processed into pet food.

Field investigation in Victoria and in the Northern Territory

Field staff from Agriculture Victoria and the Northern Territory Department of Industry, Tourism and Trade visited properties in Victoria and Northern Territory (NT), respectively, to obtain information about horse sales and movement between properties, and to collect blood samples from cohort horses still resident on the NT property.

Table 1. List of aetiological agents that are known to be associated with canine liver disease and were initially considered as differential diagnoses for cases presenting in East Gippsland in June 2021

Infectious	Bacterial	*Leptospirosis spp.*
		Yersinia pseudotuberculosis[¶]
		Clostridium piliforme (Tyzzers disease)[¶]
		Nocardia asteroids[¶]
		Mycobacteria spp.
		Helicobacter canis[¶]
		Enterobacteriaceae (infection via bile duct)
	Viral	Canine adenovirus 1 (infectious canine hepatitis)[¶]
		Canid herpesvirus 1[‡]
	Parasitic	Canine babesiosis
		Ehrlichia canis (canine monocytic ehrlichiosis)
		Cestodes (e.g., *Echinococcus multilocularis*[†]), nematodes (e.g., *Capillaria hepaticum*[¶]) or trematodes (e.g., *Herobilharzia Americana*[†], *Metorchis conjuctus*[†], *Opisthorchis felineus*[†], *Clonorchis sinensis*[†])
		Neospora caninum[§]
		Hepatozoon canis[¶]
		Hepatic coccidiosis[¶]
	Fungal	*Histoplasma capsulatum*[¶]
		Cryptococcus spp.[§,¶]
		Coccidiodes immitis[†]
		Sporothrix schenchkii[‡,§,¶]
		Aspergillus spp.
		Prototheca spp. (algae)[§]
Toxic	Chemical toxins	Cleaning agents (e.g., phosphoric acid, quaternary ammonium compounds, carbon tetrachloride, phenols, pine oil)
		Semi-volatile organic compounds (e.g., solvents)
		Free radical toxicants (e.g., iron, copper)
		Some herbicides or pesticides
	Plant-based toxins	Indospicine (*Indigofera spp*) including secondary ingestion of contaminated meat
		Fluoroacetate from plant sources (e.g., *Gastrolobium spp, acacia spp)*
		Methylazomymthanol (cycads/zamia spp)[‡]
	Fungal toxins	Wild mushrooms (e.g., *amanita phalloides*, *phallus spp*.)
		Aflatoxins B1, B2, G1, G2, M1 (mainly produced by *aspergillus flavus, A. parasiticus*)
		Other mycotoxins (e.g., phomopsins, sporidesmin, penitrem A)
	Baits	Vertebrate pest baits (e.g., sodium fluoroacetate [1080], pindone, sodium nitrate)
		Rodenticides (e.g., zinc phosphide, difethialone, brodifacoum)
	Other	Drug induced liver injury (e.g., beta-lactams, sulfonamides, statins, pentobarbital, non-steroidal anti-inflammatories, anticonvulsants, ketoconazole etc.)
		Xylitol (artificial sweetener)
		Cyanobacteria (microcystin spp)

[†] Agent is exotic to Australia.
[‡] Typically has a tropical distribution.
[§] Usually associated with other or additional clinical signs.
[¶] Rare or sporadic occurrence.

Results

Substantiated cases

Most cases were referred directly to Agriculture Victoria by private veterinary practitioners. Initially, cases were identified only from East Gippsland but later in the event also from the La Trobe Valley area of Gippsland, bayside suburbs in south-east Melbourne, and from around the Macedon Ranges. Other cases were notified to the investigations team through the PetFast network – an online notification portal for private practitioners established by the Australian Veterinary Association and the Pet Food Industry Association of Australia (PFIAA) to identify health problems potentially associated with pet food), or other informal channels.

Figure 1. Geographic distribution of substantiated cases of canine hepatopathy in Victoria between June and September 2021

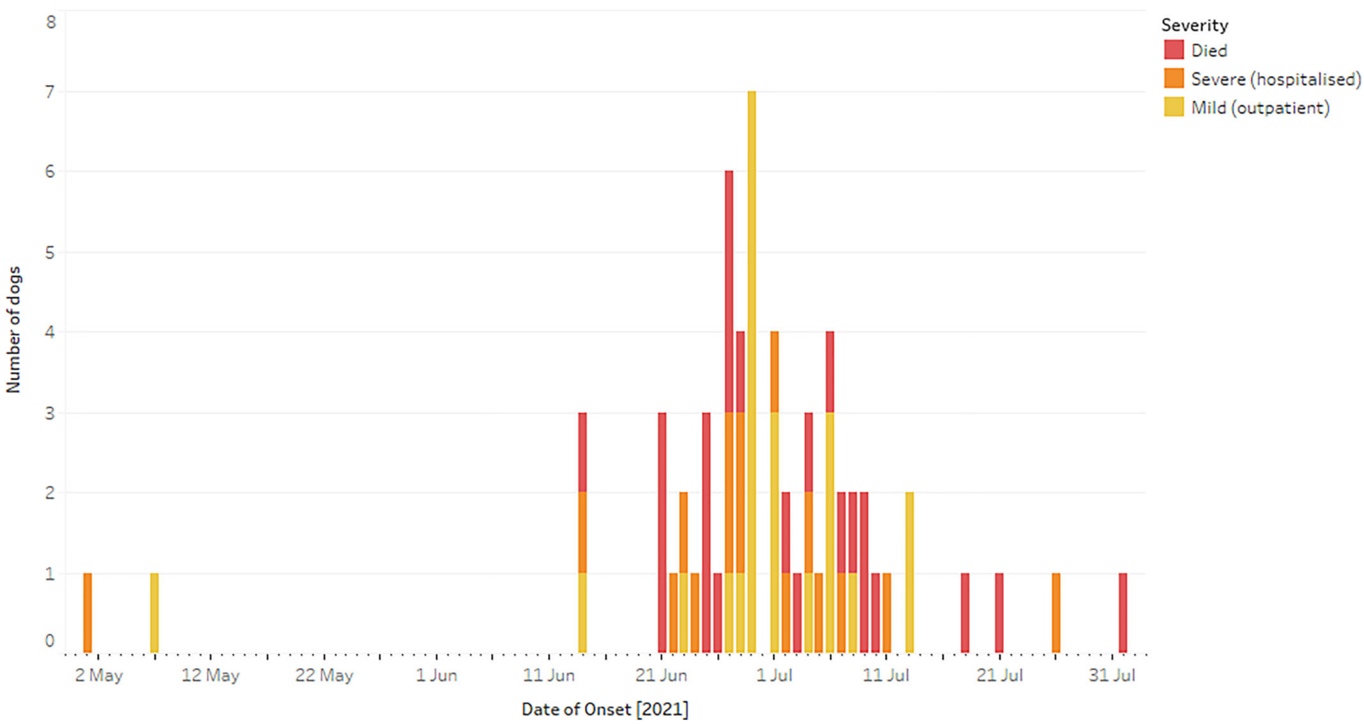

Figure 2. Date of onset and severity of clinical signs of canine hepatopathy in Victoria in 2021

By 27 August 2021, 64 canine cases meeting the case definition, including 24 with fatal outcomes, from a total of 44 households, and 25 veterinary clinics, had been reported and their details substantiated. The investigation team was made aware of a further nine anecdotal cases that included seven with apparently fatal outcomes but for which the dog owners and/or their veterinary practitioners were unable to provide further case details.

The substantiated cases were concentrated around Bairnsdale, along the Princes Highway towards Melbourne, and around the bayside and the north-eastern suburbs of Melbourne (Figure 1). A single case was identified in South Australia but is mapped in East Gippsland as this was a young dog normally resident in East Gippsland that had travelled to South Australia with its owners and while there presented with signs of hepatopathy. Another single case of canine hepatopathy was identified in western Victoria and although the investigating team does not believe that case is connected to the main cluster having very different exposures from all the other cases, it was retained in the study data as it met the case definition.

The date of onset of clinical signs was available for all except three dogs. The earliest date of onset of clinical signs in a substantiated case was 1 May 2021, and the latest was 1 August 2021 (Figure 2). Most owners reported clinical signs from 14 June onwards (Figure 2). The clinical signs reported by owners of cases were variable, with severity classified as either subclinical (dogs which had elevated liver enzymes without clinical signs) ($n = 2$), mild (affected dogs required outpatient treatment of symptoms only) ($n = 22$), severe (requiring hospitalisation) ($n = 16$) or death ($n = 24$), (Figure 2). The first reported death linked to this cluster occurred on 24 June and the last recorded was on 23 August.

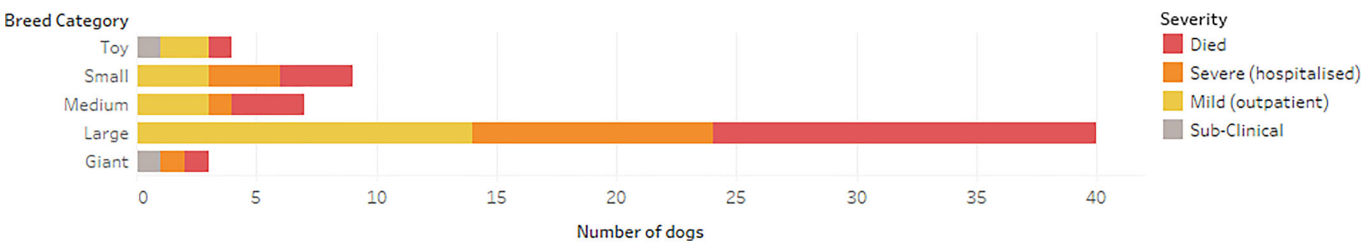

Figure 3. **Breed category and severity of clinical signs in substantiated cases of canine hepatopathy in Victoria between June and September 2021. Approximate weight ranges: Toy up to 7 kg; small 7–12 kg; med 12.1–25 kgs; large 25.1–45 kgs; Giant 45kgs plus.**

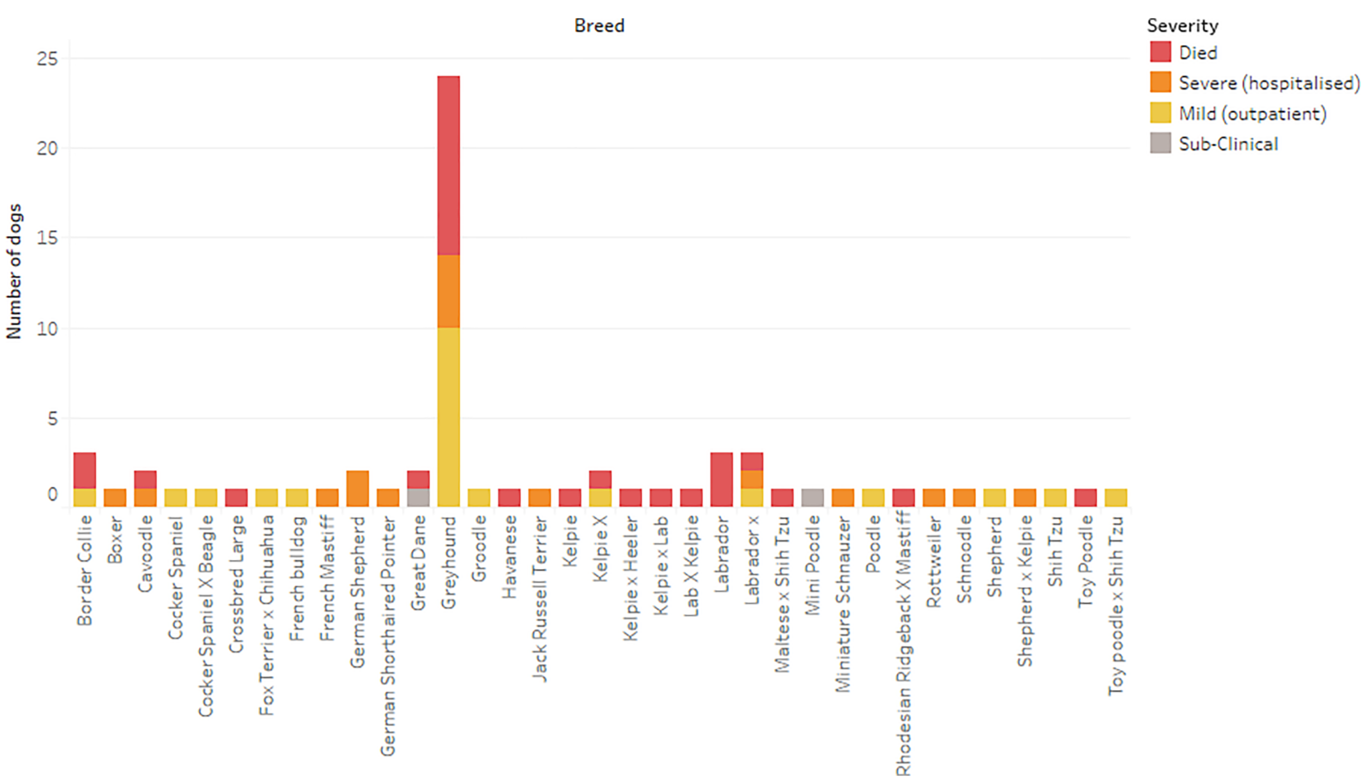

Figure 4. **Breed and severity of clinical signs in substantiated cases of canine hepatopathy in Victoria between June and September 2021**

The mean age of affected dogs was 7.5 years (range 7 months–16 years), and the mean number of dogs per affected household was 1.45 (range 1–7). Exactly 67% of affected dogs were female and 91% of affected dogs were vaccinated. Many different breeds of dogs were affected, but large dogs (25.1–45 kg bodyweight), including notably, greyhounds, were over-represented in both cases and deaths (Figures 3 and 4).

Food products

The most common food source identified amongst households reporting one or more cases of canine hepatopathy was the feeding of raw or cooked meat products either supplied directly by the local knackery or through retailers supplied by the knackery ($n = 38$, 86.4%). Owners of affected dogs reported feeding a range of different product lines including chopped/diced/minced meat products, cooked or raw products and meat products blended with rice, other grains and/or vegetables. Identification of the specific products or batches fed was limited by owner recollection of product purchased, repackaging and aliquoting by owners, and absence of informative labelling on supplied product. Products were grouped by protein source identified by owner (Table 2). No owners indicated that they were aware that the product they were purchasing may have contained horse meat.

PrimeSafe investigation

Both PrimeSafe and Agriculture Victoria maintained contact with the operator of the knackery and the parties exchanged verbal and written correspondence throughout the investigation. A total of six inspections of the knackery and various retailers and wholesalers were undertaken by PrimeSafe investigators and 14 separate statements were provided by the knackery to investigators between 19 July and 6 August 2021. In summary, investigations revealed that no camel meat had ever been incorporated into the knackery's product. Horse meat was a regular inclusion and was obtained from local (Gippsland) sources when available and from a livestock dealer. Investigations revealed that 26 horses originating from an area in the NT, where the toxic Indigofera plants are known to grow, had been transported into Victoria on 16–17 May 2021 and all were processed by the knackery between 25 May and 8 June 2021.

Under current regulation in Victoria, product recalls for pet food products cannot be enforced, but voluntary withdrawal of some product lines was undertaken by some retailers and the knackery, after consultation with PrimeSafe Officers, as the investigation proceeded.

Clinical pathology

Copies of clinical pathology results undertaken in-house (IDEXX Catalyst Dx Chemistry Analyzer) by private practitioners were provided for 10 cases. Coincidentally, the clinics all used an IDEXX analyser, so the results from different clinics could be collated. The main

Table 2. Knackery meat product types fed to substantiated cases of canine hepatopathy, as reported by dog owners

Knackery product fed	Number of cases (%)
Beef	28 (63.6)
Kangaroo	9 (20.5)
Chicken	2 (4.5)
Venison	0 (0)
Unknown	5 (11.4)

Many dogs were fed several different products and data are not available for all cases.

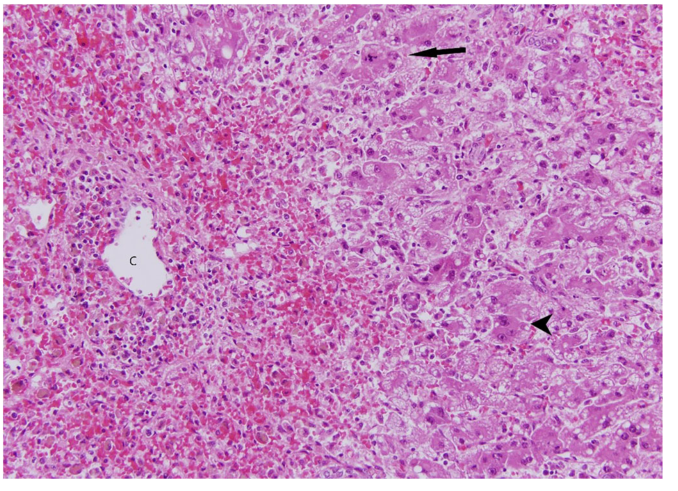

Figure 5. Histopathology (H&E) of the liver of a substantiated case of canine hepatopathy showing loss of centrilobular hepatocytes with replacement haemorrhage. The terminal hepatic venule (C) is surrounded by haemosiderin-laden macrophages and fewer lymphocytes. Midzonal and periportal hepatocytes are disorganised and have cytoplasmic vacuolation, occasional mitotic figures (arrow) and multinucleation (arrowhead).

Table 3. Clinical pathology results from 10 dogs, undertaken on in-house (IDEXX Catalyst Dx Chemistry Analyzer) by private practitioners in East Gippsland, Victoria in 2021

Parameter	Number of samples	Mean value	Min/max values from tested samples	Normal canine value range
ALT	6	1722 IU/L	138/3361 IU/L	10–125 IU/L
ALP	10	435 IU/L	117/1550 IU/L	23–212 IU/L
GGT	9	36 IU/L	18/103 IU/L	0–11 IU/L
Total bilirubin	10	164 μmol/L	20/693 μmol/L	0–15 μmol/L

biochemical changes observed included a marked (>10-fold) increase in ALT, greater than 15-fold elevation in bilirubin, and elevations of ALP and GGT concentrations (Table 3).

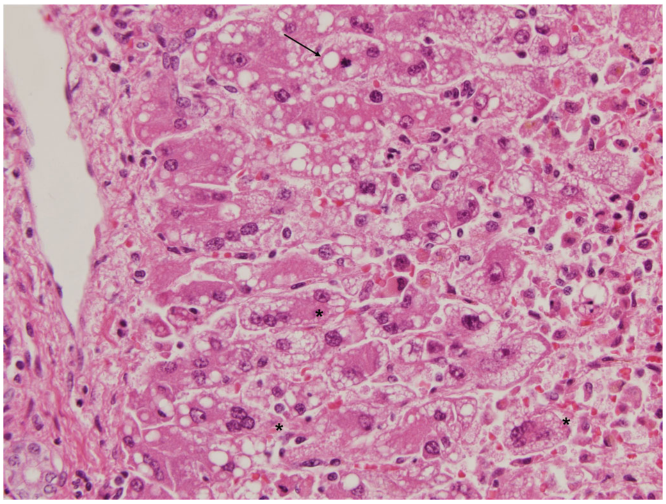

Figure 6. Midzonal and periportal hepatocytes exhibit cytoplasmic vacuolation, multinucleation (asterisks) and mitotic figures (arrow). H&E.

Gross pathology

Typical gross pathological changes reported by attending practitioners included generalised icterus, and that the livers of affected dogs were small and congested, with an enhanced zonal pattern seen as alternating cream and dark red coloured regions observed on the capsular and cut surfaces.

Histopathology

Fixed tissues were submitted to Veterinary Diagnostic Services – AgriBio from nine dogs between 3 July and 17 August. Histopathological lesions in the cases submitted in early July consisted of diffuse loss of periacinar hepatocytes with pooling of blood in haphazardly arranged microvasculature (severe periacinar necrosis – Figure 5). The presence of numerous macrophages with ample cytoplasmic golden-brown pigment (haemosiderin) was a prominent feature and there were variable (absent to moderate) infiltrates of lymphocytes surrounding the central veins. The remaining midzonal and periportal hepatocytes were markedly disorganised and exhibited distinct cytoplasmic vacuolation (small and multiple), anisocytosis, anisokaryosis with frequent multinucleation (see Figure 6) and mitotic figures. Submissions received in late July to mid-August lacked the prominent hepatocellular anisokaryosis, multinucleation and mitoses, though periacinar changes were consistent with earlier cases with the addition of variable periacinar to bridging fibrosis and biliary hyperplasia, features which indicate chronicity.

Table 4. Indospicine levels in serum and/or liver from substantiated cases of canine hepatopathy, and also in the pet meat consumed by those dogs (where that was available for testing)

Case	Date dog died	Indospicine level in dog serum	Indospicine level in dog liver	Indospicine level in pet meat	Pet meat speciation
1	1 July 2021	3.46 mg/L	4.34 mg/kg	< limit of quantitation	Not tested
2	1 July 2021	2.61 mg/L	5.03 mg/kg	Not tested	Not tested
3	11 July 2021	No sample available	1.55 mg/kg	Not tested	Not tested
4	13 July 2021	1.45 mg/L	2.28 mg/kg	< limit of quantitation	Beef, kangaroo, horse positive
5	23 July 2021	1.72 mg/L	Sample held but not tested	11.75 mg/kg[†] and 0.14 mg/kg[‡]	Beef, kangaroo, horse positive
6	29 July 2021	1.44 mg/L	2.01 mg/kg	0.18 mg/kg	Beef, kangaroo, horse positive
7	2 August 2021	2.94 mg/L	Sample held but not tested	0.17 mg/kg	Beef, kangaroo, horse positive
8	23 August 2021	1.78 mg/L	Not sampled	106.60 mg/kg[§]	Beef, kangaroo, horse positive
9	23 August 2021	1.38 mg/L	Not sampled	0.12 mg/kg	Beef, kangaroo, horse positive
10	Dog remains alive (as 23 August 2021)	1.39 mg/L	No sample available	0.21 mg/kg	Beef, kangaroo, horse positive
11	Dog remains alive (as 23 August 2021)	0.06 mg/L	No sample available	Not tested	Not tested
Mean		*1.82 mg/L*	*3.04 mg/kg*		

[†] Purchased 22 June 2021.
[‡] Purchased before 17 July 2021.
[§] Purchased 15 June 2021 from a retail outlet.

SMALL ANIMALS

Table 5. **Results of indospicine testing on dog food samples provided by the owners of substantiated cases of canine hepatopathy where the dogs were not tested for indospicine exposure**

Submitter	Sourced from	Purchase or retention date	Indospicine level in pet meat	Pet meat speciation
Dog owner	Direct sale ex knackery	2 Jun 21	4.87 mg/kg	Beef, kangaroo, horse positive
Dog owner	Retailer	2 Jun 21	0.38 mg/kg	Beef, kangaroo, horse positive
Dog owner	Retailer	8 Jun 21	0.15 mg/kg	Beef, kangaroo, horse positive
Dog owner	Direct sale ex knackery	9 Jun 21	1.05 mg/kg	Beef, kangaroo, horse positive
Dog owner	Direct sale ex knackery	9 Jun 21	0.08 mg/kg	Beef, horse positive
Wholesaler	Wholesaler	9 Jun 21	0.15 mg/kg	Beef, kangaroo, horse positive
Wholesaler	Wholesaler	9 Jun 21	0.23 mg/kg	Beef, kangaroo, horse positive
Wholesaler (2 samples)	Wholesaler	9 Jun 21	<limit of quantitation	Beef, kangaroo, horse positive
Wholesaler (6 samples)	Wholesaler	9 Jun 21	<limit of quantitation	Beef, horse positive
Dog owner	Direct sale ex knackery	15 Jun 21	4.49 mg/kg	Beef, kangaroo, horse positive
Dog owner	Retailer	15 Jun 21	6.29 mg/kg	Beef, kangaroo, horse positive
Dog owner	Retailer	17 Jun 21	12.98 mg/kg	Beef, kangaroo, horse positive
Knackery retention samples	Knackery	17 Jun 21	<limit of quantitation	Beef, kangaroo, horse positive
Knackery retention samples	Knackery	24 Jun 21	<limit of quantitation	Beef, kangaroo, horse positive
Knackery retention samples ×1	Knackery	1 Jul 21	<limit of quantitation	Beef, horse positive
Dog owner	Direct sale ex knackery	5 Jul 21	0.18 mg/kg	Beef, kangaroo, horse positive
Knackery retention samples	Knackery	8 Jul 21	<limit of quantitation	Beef, kangaroo, horse positive
Knackery retention sample – kangaroo	Knackery	14 Jul 21	<limit of quantitation	Beef, kangaroo, horse positive

Table 6. **Indospicine test results from equine blood samples obtained from three live horses from the same source property in the Northern Territory as horses processed at an eastern Gippsland knackery during the 2021 outbreak of canine hepatopathy**

Sample	Indospicine level in serum
Horse 1	41.95 mg/L
Horse 2	14.81 mg/L
Horse 3	17.65 mg/L

Exclusion of infectious causes

Agent-specific testing was performed to exclude leptospirosis (fresh urine qPCR) on six cases and canine monocytic ehrlichiosis (EDTA blood for *Ehrlichia canis* qPCR, serum for antibody ELISA) for four cases. All returned negative results.

Toxicology results

Total aflatoxin (measured through ELISA) was found to be <1.0 ppb (below the level of detection) for two feed samples submitted, and that toxin was therefore excluded as a potential cause of the observed hepatopathy cases. Indospicine was detected in all three canine liver and serum samples initially analysed, and the toxin was eventually detected in samples from 11 dogs and 18 pet food aliquots sampled (Tables 4 and 5).

Indospicine levels in horses

There were no horses remaining alive and available for testing from 16 to 17 May 2021, NT consignment. However, serum samples were collected from three cohort horses still resident on the property of origin in the NT. All three animals showed high levels of indospicine (Table 6).

Discussion

This paper outlines the steps in the investigation to accumulate sufficient information and high-quality samples to enable the cause of the observed hepatopathy to be identified.

From the outset, reports of liver dysfunction amongst carnivorous domestic pets other than dogs were not excluded from the investigation, and data were obtained on the details and health status of other pets kept by both case and control households. Despite extensive media communications and high public interest throughout this investigation, there were no indications that the observed cluster of hepatic disease extended to domestic animal species other than dogs. Two reports of illness in cats were investigated during the study period. Both cases were excluded due to comorbidities and insufficient diagnostic testing to confirm acute onset liver disease.

To the best of our knowledge, estimates of the "normal" or "expected" incidence of canine hepatopathy in Victoria have not been reported. This presents a challenge to the identification of new or emerging disease clusters as the normal background level of liver disease in dogs remains unknown. In this incident, the scale of the event ensured its rapid identification by private veterinary practitioners (and subsequent notification to Agriculture Victoria) as an unusual disease event. This then facilitated strong and early interventions, like broad notification of veterinarians state-wide, and limited product recalls aimed at preventing additional cases.

The identification of subsequent cases was also supported through PetFAST. During this incident, provided the notifying veterinarian authorised release of their contact information, suspect cases of canine hepatopathy identified through the PetFAST system were forwarded to Agriculture Victoria for investigation, providing a convenient platform for information gathering during a known incident. Noting that the system is available for veterinarians irrespective of their AVA membership status, this mechanism could potentially be adapted to assist with the identification of emerging non-feed-related disease events in the future. Limitations of the system include the inability to follow up cases where owners/practitioners request anonymity meaning that a few potential cases could not be confirmed, and that feedback to industry was available only to members of the PFIAA, which excludes significant sections of the pet food sector, including knackeries.

Despite early anecdotal evidence suggesting that pet meat originating from a single knackery may have been linked to the outbreak of canine hepatopathy reported, differential diagnoses including infectious disease, poisoning (including environmental, accidental, malicious or blue-green algae) or contaminants in feed were considered and assessed through collection of thorough case histories relating to both sick and unaffected dogs in affected households, and via laboratory examination of clinical samples. The long list of possible causes (Table 1) was rapidly reduced once the initial results of laboratory testing for ehrlichiosis (recently diagnosed in a small number of dogs in Victoria) and leptospirosis (a risk with an increased rodent population following high rainfall that ended a period of drought conditions) returned negative results. Initial histopathology results were obtained, which showed a hepatopathy suggestive of a toxicosis. Subsequently, the focus on further investigations included (but was not limited to) four known toxic agents: amatoxin, aflatoxin, blue-green algal poisoning and indospicine toxicity.

Amatoxicosis, (poisoning with amanitin-containing hepatotoxic mushrooms[11]) was considered unlikely to present as an outbreak involving many dogs across a wide region simultaneously. While definitive toxicological testing was not available, it was excluded as a likely differential diagnosis owing to the more peracute and significant nature of typical histopathological lesions caused by this toxin. Aflatoxicosis has previously been linked to pet food-linked hepatopathy,[12] but outbreaks in dogs are usually linked to a carbohydrate, grain or nut component in commercial feeds; no such link could be made in this case, and preliminary testing on two samples returned a negative result. Blue-green algal poisoning (specifically *Microcystis aeruginosa* toxicosis) is associated with warmer weather and drinking, swimming or other water sports in waters affected by an algal bloom.[13] No such blooms were reported in the areas where the dogs normally lived or their owners reported travelling to, and there were no consistent reports in case histories of access to any single or potentially suspect water source (almost all case dogs lived in households using town water and only 14% of affected dogs lived on rural properties). Indospicine toxicity was therefore considered the most likely cause of the events reported, with initial histopathogical findings, and results of subsequent toxicology testing, consistent with previous reports of clinical disease from both Western Australia[4] and the NT.[3]

Initial exploratory testing of sera and liver samples from three affected dogs was undertaken, and indospicine levels were found in both sera and liver from all three dogs (Table 4) and the levels detected were comparable with indospicine levels reported in dogs that had consumed indospicine-contaminated camel meat.[4] Despite serum and liver samples being consistent with indospicine toxicity, the initial selection of pet food samples provided by the owners of the same three affected dogs did not contain detectable indospicine. This suggested that the presence of the indospicine-contaminated meat would not be uniform and that samples from feed offered between 2 and 4 weeks prior to the date of onset of clinical signs should be the next subject of testing (*M Fletcher, pers comm*). An expanded number of pet food samples including earlier date ranges was then tested, with indospicine detected at variable and sometimes very high levels (Tables 4 and 5). Of particular note, four different pet food samples provided by dog owners and reportedly all purchased on 15 June from either retail outlets or the knackery were shown to have very high levels of indospicine, including one sample that contained 106.60 mg/kg indospicine, a level that exceeds concentrations reported in any previous report of hepatopathy in dogs.

Following the identification of indospicine as the likely toxic agent, meat speciation was prioritised to identify a potential common source. This testing confirmed the presence of horse meat in all the samples examined, despite owners being unaware of its presence in the pet meat they had regularly purchased for their pets. The presence of horse meat in all samples was considered significant, owing to the previously documented incident in the NT involving canine liver disease following indospicine-contaminated horse meat.[3] Investigations at the knackery were subsequently focused on processed horses as the likely source of the contamination, culminating in the detection of a plausible and substantiated account of the movement of 26 horses from the NT. This was further validated through serological testing of cohort horses from the property of origin in the NT, which were found to have high serum indospicine levels (Table 6).

Conducting a case–control study complemented the investigation and supported the rapid verification of a link between pet meat sourced from a single knackery and canine hepatopathy, even while definitive toxicology results remained pending.[9] The observed high incidence of toxicosis in greyhounds was explored in the case–control study and found to be associated with a general increased risk for large breed dogs. This, and a likely increased preference by greyhound owners to feed knackery pet meat, may explain their over-representation in the event.

As testing of both dogs and feed samples progressed, it became clear that there was very high variability in the level of indospicine toxin detected in all sample classes. Considering pet meat, it is likely that there was nonhomogeneous distribution of the toxin in the raw feed; potentially high contamination in some batches, but with batch-to-batch variation in the product throughout the period from end of May to early July. Added to this, we recognised that there was on-processing and blending of the pure meat product by wholesalers and retailers in the supply chain, sampling variability and additional sources of variability linked to the dogs (feed preferences, feeding rate, length of time on the feed) all contributing to the variation seen in test results. This led to challenges for the investigation team who were aiming to provide a consistent and accurate communications message to owners, veterinarians, the knackery and pet food retailers about possible risk products and resulted in the need for conservative estimates for relevant risk periods and products.

After reviewing the information that was available, a risk period for pet food produced between 31 May and 3 July 2021 was set, based initially on likely dog exposure commencing 2 weeks before the onset of clinical signs in mid-June, and the date when product was recalled by some retailers (3 July 2021). The risk period was reviewed regularly through the event but remained unchanged. All affected retailers were actively recalling product by 19 July, but we recognised that some contaminated feed may have remained in the supply chain and in storage in home freezers. For this reason, the message was regularly reinforced, and when we were advised of an owner feeding contaminated product under the misguided (and sadly fatal) assumption that cooking would remove the toxin, the messaging was augmented with warnings that all meat products from the knackery should be considered a risk and that neither cooking nor freezing would remove the toxic agent.

This report outlines the sequence of events and the thought processes that guided what was from the outset, a disease investigation rather than a clinical trial or research project. It was unclear whether as a largely agriculture-focused government animal health agency, Agriculture Victoria, had a role in investigating an outbreak of disease in dogs, but there clearly was a role for government in facilitating testing locally and nationally and expanding the investigation across the state. The data presented represent the cases that were notified to Agriculture Victoria and could be substantiated. The aim was to find and document as many cases as would be required to understand the aetiology of the outbreak, not necessarily to find and document every case. Similarly, not every case underwent testing with a full panel of diagnostic or toxicological screening tests. Testing was conducted to rule in and out lines of investigation and was in every case reliant on samples being made available by the dog's owner and their vet. A major omission from the study, which reflects these limitations, was the lack of a full necropsy on any affected dog; no owner consented to the procedure, but in the end, it made little difference to the outcome and effective use was made of liver biopsy specimens carefully and respectfully collected, with owner's permission from deceased pets.

The investigation deviated from those generally undertaken in that it was conducted during periods of significant public health restrictions due to the SARS-CoV2 pandemic. The logistics of collecting and transporting samples were challenging, as were securing supplies of laboratory reagents and ensuring access to laboratories for testing. These issues were highlighted as staff tried to negotiate the different regulations in place simultaneously in four jurisdictions to enable sample collection in Victoria, South Australia and the NT, then transport to, and testing in, laboratories in Victoria, Queensland and South Australia. Delays in the investigation ensued and caused frustration for all parties.

Another significant feature of the investigation was the extensive public interest generated through social and traditional media. This was successfully utilised to assist in identifying potential cases, alerting dog owners and vets to the investigation underway, and to source control households for the case–control study. The response to social media posts was extraordinary, with 40,000 views and 20,000 shares between 3 and 10 July. For appropriate subject matter, this medium is a very powerful tool.

In Victoria, knackeries are licensed meat processing facilities that produce pet food, skins and hides and other by-products from fallen, terminally unwell or unwanted livestock. These processing sites fulfill an important role in managing end-of-life welfare for livestock and protecting the environment by providing a managed off-farm carcass disposal process. Product labelling, safety and product recall/withdrawal processes for pet food do not mirror those applied to meat for human consumption, despite the public's assumption that they should; even under the circumstances outlined in this case, there was no way of requiring or enforcing a product recall from any pet food manufacturing site or retail outlet. In addition, it was impossible to determine from the product labelling, the manufacture date or meat composition in any of the pet meat samples purchased directly from the knackery or provided to their wholesalers. Pet meat was reportedly purchased by owners in large (5 kg, 10 kg or 20 Kg) unlabelled bags or buckets. In many cases, there was no record of the transaction. The pet food manufacturing standard[10] requires basic records to be kept by pet meat manufacturers, but the policy basis of the standard is to prevent pet meat entering the human food supply chain, not to ensure pet safety or the ability to recall product. Consequently, when these asymmetric expectations around standards were revealed, the case attracted much interest and passion from pet owners, the media, the veterinary profession and other pet food processors, resulting in re-ignition of the national conversation on regulation of pet food. In the meantime, knackery operators are initiating their own risk management strategies in a genuine attempt to prevent further incidents like the one described here and PrimeSafe is implementing enhanced livestock traceability requirements for knackeries.

This investigation also highlighted many issues relating to the lack of horse traceability at a national or state level. Under the *Livestock Control Act 1994*, horse owners must apply to the Secretary of the Department of Jobs, Regions and Precincts for the allocation of a Property Identification Code (PIC) that identifies the place at which the horse or horses are to be kept. Likewise, a person who carries on a livestock business (e.g., an abattoir or knackery) must apply to the Secretary of the Department of Jobs, Regions and Precincts for the allocation of a code that identifies the place at which the person carries on that business. Unlike some other livestock industries, however, there are no PIC recording requirements for horses being moved into or within Victoria. Consequently, there are no reliable estimates of the number of horses sold or sent to slaughter annually.[1] Anecdotal reports suggest that the pattern of horse sales in Victoria has changed since March 2020, largely due to the imposition of restrictions to manage the spread of the SARS-CoV2 virus leading to reduced (or largely non-existent) sales of local horses for slaughter. These changes may have contributed to the need for the direct sale of horses from the NT to the Victorian knackery that led to the events recorded here. In the absence of a horse traceability database or even a paper trail of recorded movements, it was impossible to independently verify the movement of the horses central to this event. Without the cooperation of the knackery ownership and the livestock trader, we may not have reached any understanding of the sequence of events leading to the dog's illness.

Conclusions

Two months after the commencement of the investigation into a cluster of severe canine hepatopathy cases, a substantiated account of the movement of 26 horses from the NT to Victoria had been collated. The horses, processed over a period of 14 days, were the most plausible source of the indospicine toxin in the pet meat, which caused the death of 24 dogs and severe illness in at least 40 other affected dogs. This is the first report of severe and frequently fatal hepatopathy in dogs in Victoria relating to consumption of pet meat contaminated with indospicine.

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[1] The horse slaughter sector is comprised of abattoirs that slaughter horses for human consumption and knackeries that receive animals for pet food processing. Horses may not be slaughtered for human consumption in Victoria and horse meat may not be brought into Victoria for sale for human consumption.