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Author/s:

Chen, T;Hufschmid, J;Whiteley, P;El-Hage, C;Davis, N;Skerratt, LF

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## ORIGINAL ARTICLE

# Chronic phalaris toxicity in macropods is widespread and peaks in July in Victoria, Australia

T Chen,<sup>a\*</sup> J Hufschmid,<sup>a</sup> P Whiteley,<sup>a</sup> C El-Hage,<sup>a</sup> N Davis<sup>b,c</sup> and LF Skerratt<sup>a</sup>

*Phalaris aquatica* is pasture species introduced into Australia during early European settlement. Consumption of the plant can cause the neurological condition chronic phalaris toxicity (CPT) in sheep and cattle. In recent years, there has been an increase in reports of CPT in macropods, which has raised concerns regarding its impacts on their welfare. Currently, little is known about the distribution or seasonal patterns of this disease in wildlife, information pivotal in assessing its potential risks. Between 2021 and 2022, we conducted a survey targeting government bodies, veterinary businesses and wildlife organisations to investigate the locations and time of occurrence of CPT in macropods in the state of Victoria, Australia. We received 13 survey responses, 12 verbal reports, a full record of investigated cases from a university veterinary school and cases from a wildlife rescue organisation. Over the period of 11 years, Victoria had 918 cases of CPT recorded in macropods from 36 local government areas, with cases concentrated centrally just north of the state capital of Melbourne and July (midwinter) being the month with the highest case count ( $n = 220$ ). There was a significant positive correlation between case count and both the abundance of kangaroos (*Macropus giganteus* and *Macropus fuliginosus*) ( $P < 0.01$ ) and the abundance of *P. aquatica* ( $P = 0.009$ ), and a significant negative correlation between annual case count and average rainfall of March ( $P = 0.016$ ) and April ( $P = 0.02$ ). Understanding these relationships will assist land and wildlife managers in predicting the risk and magnitude of disease outbreaks of CPT each in Victoria.

**Keywords** chronic; macropod; Phalaris; spatial; temporal; toxicity

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Chronic phalaris toxicity (CPT) is a neurological disease that was first identified in Australia in 1942 when a flock of sheep was exhibiting ‘stagger’-like signs after grazing a paddock of *Phalaris aquatica*.<sup>1</sup> *Phalaris aquatica* is a perennial pasture plant that was introduced into Australia in the early 1900’s from the Mediterranean region for its high winter production and drought resistance.<sup>2,3</sup> Since then, multiple cultivars have been developed for improved agriculture production, and the pasture species has seen

wide usage in the south-eastern states of Australia including South Australia, Victoria, New South Wales and Tasmania.<sup>2,4</sup> *Phalaris* pasture has since, to a lesser extent, also been introduced in New Zealand, USA, Europe and the African continent.<sup>5</sup> Consumption of *P. aquatica* causes two distinct diseases in grazing livestock known as acute phalaris toxicity and CPT. The former is speculated to be related to disruptions to the ammonia balance leading to a syndrome of sudden death,<sup>6,7</sup> whereas the latter is caused by a group of naturally occurring tryptamine alkaloid toxins produced by the plant in the early phases of the growth period.<sup>8,9</sup> The toxins, which are serotonin analogues, are capable of causing overstimulation of serotonergic receptors.<sup>7</sup> The resulting clinical presentations of animals are chronic and include ataxia, hyperexcitability, muscle tremors that can be localised to the face or full body, hypermetria, collapse and exhaustion.<sup>1</sup> At postmortem examination, gross dark grey discoloration of the brain is observed, which presents as brown pigment deposits within neuron cytoplasm on histopathology.<sup>10</sup> CPT primarily affects sheep, but cattle can also be affected,<sup>1,11</sup> and there have been reports of cases of other *Phalaris* species causing CPT-like signs in horses.<sup>12,13</sup> There are currently no reports of wildlife toxicities from any *Phalaris* species outside of Australia.

CPT in Australian endemic wildlife was first reported in 1983, when lesions in red kangaroos (*Macropus rufus*) were briefly described as being similar to those seen in sheep after having grazed on *Phalaris* pasture.<sup>14</sup> The clinical presentation and histopathological changes were only recently examined in detail, confirming the diagnosis in macropods.<sup>15,16</sup> The clinical signs described in affected macropods include incoordination, a wide based gait, hypermetria and muscle tremors, with brown pigmentation of the neurons, similar to that of sheep, seen histologically.<sup>15</sup> Several significant outbreaks were reported in 2018 and 2019, particularly in the state of Victoria. The number of animals affected per site ranged from 30 to 100 animals, with many of them euthanised after being found in severe stages of disease (Parks Victoria: K Moran and S Mundy, personal communication, 2021). All outbreaks recorded in these 2 years affected eastern grey kangaroos (*Macropus giganteus*), but the disease has also been seen in western grey kangaroos (*Macropus fuliginosus*), red kangaroos (*M. rufus*), tammar wallabies (*Notamacropus eugenii*) and Bennett’s wallabies (*Notamacropus rufogriseus*).<sup>14–16</sup> Incidents of CPT have since gained increased public attention and raised concern about the impacts of the disease on animal welfare and public safety around affected animals.

Insights into the spatial and temporal occurrence of CPT in macropods are currently very limited. Cases have been reported on

\*Corresponding author.

<sup>a</sup>The University of Melbourne, 250 Princess Highway, Werribee, Victoria 3030, Australia; t.chen093@gmail.com<sup>b</sup>Parks Victoria, Level 10, 535 Bourke St, Melbourne, Victoria 3000, Australia<sup>c</sup>School of Biosciences, The University of Melbourne, Parkville, Victoria 3052, Australia

Kangaroo Island, South Australia, and the rural suburbs of Seymour, Dartmoor and Axedale in Victoria, with cases occurring anytime between January to November,<sup>15</sup> and in Tasmania near Hobart between April and October.<sup>16</sup> However, the information provided by these reports probably underrepresents the true spatial and temporal distribution, as news reports, recent personal communications with rescuers and others who have witnessed the disease suggest that the disease occurs in a much wider area during various times of the year. Specific knowledge on the distribution and seasonal occurrence of CPT in macropods is vital to future management including disease prevention strategies.

Compared with macropods, reports of CPT in livestock show greater consistency in the temporal patterns of disease, with the disease mostly occurring in late autumn and winter.<sup>7,17</sup> One explanation for this timing is that *P. aquatica* produces increased levels of tryptamine alkaloids during the early growth phase after summer dormancy and can remain toxic to animals for as long as 5 months into the growth period.<sup>8</sup> However, livestock disease patterns may not be accurate predictors of CPT occurrence in macropods. Importantly, the rate of consumption of *Phalaris* differs between species. Unlike livestock, which are often restricted to *Phalaris* pasture due to farming practices, macropods generally have wider home ranges within which they can selectively graze different types of vegetation and ingest a diverse range of plants.<sup>18</sup> This may change the quantity of toxins ingested based on the availability and intake of other plants. Prediction of the distribution of CPT is also complicated by factors such as population size and pasture utilisation behaviour, which are not uniform across the landscape and are likely to influence intake of *Phalaris*. These challenges make predictions of macropod CPT patterns using agriculture models unreliable. It is therefore important to directly investigate disease patterns of CPT in wild macropods to gain an accurate depiction of the disease dynamics.

To address knowledge gaps in the temporal and spatial distribution of CPT in macropods and the environmental and demographic factors affecting these, this study aimed to collate retrospective case reports from wildlife professionals, universities and government. This information is crucial in improving our ability to predict the risk and estimate the impact of CPT on macropod welfare in Victoria. It will also allow future research and management strategies to target high-risk geographical areas or seasons.

## Materials and methods

### Data collection

Government organisations, veterinarians and wildlife management and rescue organisations in Victoria, Australia, were surveyed anonymously under Human Ethics Approval 2021-21488-18012-3 (The University of Melbourne). The survey was distributed using an online survey program (Qualtrics™, Provo, USA) from June 2021 to September 2021 (Table S1). Contact information for government organisations and departments was obtained initially through government staff members who had previously communicated with The University of Melbourne regarding CPT cases. The publicly available contact information of every local council of Victoria was obtained through official government websites. Contact information for veterinary clinics across Victoria was obtained through the publicly

accessible business record (The Yellow Pages, Thryv, Melbourne, Australia). Contact information for wildlife management and rescue groups was obtained through personal communication with wildlife rescuers and a web browser search (Google), using the following key words: 'wildlife rescue Victoria'. The survey was distributed using a web link via email, and participants were invited to further share the link within their networks. A Plain Language Statement containing basic information on CPT, the study and contact information for the authors was attached to every invitation for the survey; anyone who preferred to contact the research team directly was encouraged to do so and report their experiences. Finally, data on cases of CPT from the pathology records of the Melbourne Veterinary School were also obtained.

Survey participants were asked for details of cases of CPT they had encountered in the past. Details requested for each incident included the location, date and the presenting clinical signs of the animals presented as tick boxes. The clinical signs listed were ataxia, muscle tremors, hyperexcitability, collapse and wide stance gait as described by past studies.<sup>15</sup> Participants were also asked if a 'gold standard diagnosis' was performed, involving assessment of clinical presentation, full postmortem examination and histopathological examination of the brain. These questions were uniformly presented to all participants either through the survey or verbally, depending on which form of communication they selected. If the response was from a past pathology record, the record was checked for evidence of clinical descriptions and histopathological confirmation.

Additionally, data on the abundance and distribution of kangaroos and *P. aquatica* in Victoria, as well as the average monthly rainfall of Victoria in March, April and May, were obtained from publicly available datasets to explore possible correlations between these variables and case numbers of CPT. Kangaroo abundance data across their Victorian distribution were obtained through the Victorian State-Wide Kangaroo Abundance Report completed in 2020.<sup>19</sup> The report was produced based on data from both aerial surveys and ground line-transect distance sampling. The estimated average abundance of both eastern and western grey kangaroos combined in each Urban local government areas (LGAs) was used for the data analysis.

Data on the abundance and distribution of *P. aquatica* were obtained through the publicly available database Victorian Biodiversity Atlas<sup>20</sup> by using the key words ("*Phalaris aquatica*"). Abundance was estimated for each LGA based on the number of verified sightings of the species in each LGA across the full historical record. This was the only type of data available to estimate *P. aquatica* abundance on a state level, because detailed estimates of abundance are only available for some sites and have not been conducted at a statewide scale. The full record was used instead of a recent time period because the plant is a perennial pasture plant that is unlikely to have rapid shifts in its distribution, thus having the full record was assumed to provide a more accurate picture of distribution over the study period.

Within the kangaroo abundance report, LGAs that were considered metropolitan Melbourne did not have an estimated abundance, because these areas were regarded to have no established wild populations of kangaroos. These LGAs were Banyule, Bayside, Boroondara, Darebin, Frankston, Glen Eira, Greater Dandenong, Kingston, Knox, Manningham, Maribyrnong, Maroondah, Melbourne,

Monash, Moonee Valley, Moreland, Port Phillip, Queenscliffe, Stonnington, Whitehorse and Yarra. To match this absence of data in the LGAs, the CPT cases and abundance of *Phalaris* in these LGAs were not included in the analysis.

Finally, monthly rainfall data for the months of March, April and May for the years of the case data were obtained through the publicly available online database Scientific Information for Land Owners (SILO).<sup>21</sup> The weather data for these 3 months were of particular interest because the level of alkaloid production of *P. aquatica* may be affected by moisture stress during its early growth phase, which occurs in these months.<sup>22,23</sup>

### Data processing

The CPT cases from all sources were combined into a single dataset to give the broadest overview of the distribution and patterns of occurrence of CPT. The dataset was then classified into three tiers, based on the level of detail (specificity) in the survey responses, and each tier was analysed separately to identify any differences in findings based on the level of accuracy of case diagnosis. Tier one responses were considered the most accurate diagnosis of CPT and included confirmation of clinical signs and a histopathological confirmation. Tier two responses included descriptions of clinical signs that matched CPT but did not include histopathological confirmation. Tier three cases lacked both clinical description and histopathological confirmation but had been recorded by the reporter as CPT. Tier three cases were often in the form of case records from a logbook where no details other than the reason of attending to the animal were stated. The data of each case were then separated into a location (spatial) data and a time (temporal) data for the analysis.

For spatial data, the unit of measurement was LGA, which are administrative divisions of the state each having a distinct local government body. There is a total of 79 LGAs in Victoria. The unit of LGA was the most suitable unit of measurement because many survey responses did not include specific locations (e.g. due to privacy considerations or limited recollection of details) and listed either a suburb or a range of adjacent suburbs within the same LGA. Two survey responses reported a total of 79 cases from a cluster of adjacent suburbs which spanned across multiple LGAs without specifying how many cases in each suburb. To maintain spatial resolution at the LGA level and include these cases in the dataset, each of these cases was assigned to a random listed suburb from the cluster reported in the response, with a minimum of one case per suburb, using the random number generator function in Microsoft Excel. The LGA of that corresponding suburb was then used for the case location. The abundance and distribution data for kangaroos and *P. aquatica* were also summarised from the original databases at the LGA level. The monthly rainfall data was initially expressed as an interpolated raster layer with a grid size of 5 km × 5 km. This was translated into rainfall data per LGA using the GIS program QGIS (Open Source Geospatial Foundation, Chicago, USA, <https://qgis.org/en/site/>) to calculate the average rainfall within the vector lines of each LGA expressed in millimetres per month in each individual year. Temporal data were presented in units of months of the year.

### Data analysis

All statistical analyses were conducted using Jamovi (v2.3, Sydney, Australia, <https://www.jamovi.org/>). A chi-square goodness-of-fit test was performed on the number of CPT cases for both spatial and temporal data at each of the three tier levels and for the combined dataset. The expected frequency used for the calculations was the average number of cases per month and per LGA, respectively. For each data category (i.e. month or LGA), the z-score was obtained to determine if the category had a statistically significant ( $P < 0.05$ ) difference in the number of cases compared with other categories.

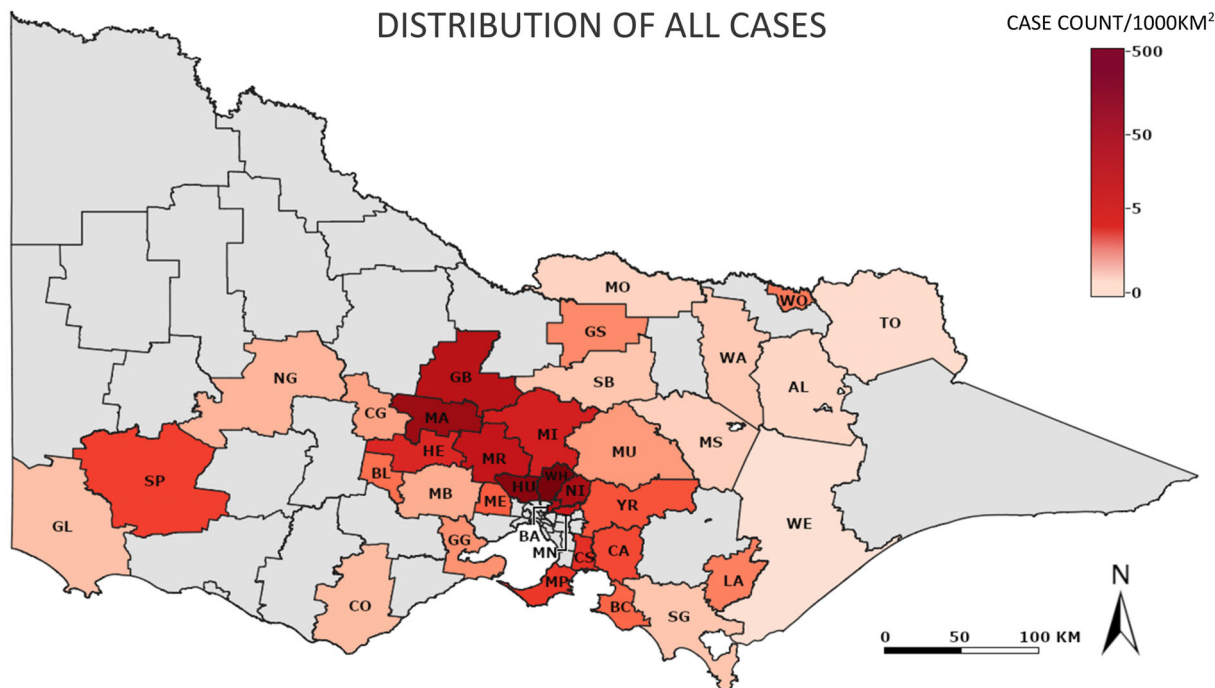
A linear regression analysis was performed at all tier levels and for the combined dataset to investigate associations between spatial occurrence of CPT and the abundance of kangaroos and *P. aquatica*. The presence of both kangaroos and *P. aquatica* are necessary causes of CPT; hence, the relationship between the abundance of both variables and the case number was analysed. All variables within the dataset were divided by the total area of their respective LGAs to standardise the case counts as well as abundance variables into unit per km<sup>2</sup>.

A linear regression analysis was also performed on the number of cases in each LGA for each specific year against the monthly rainfall of March, April and May, respectively, of that LGA in that specific year. For example, in 2018, the LGA of Mitchell had nine CPT cases, and the March rainfall of this year for Mitchell was 27 mm. The corresponding data point will have the rainfall (x-value) of 27 and a case count (y-value) of 9 for the March rainfall versus the spatial dataset analysis.

## Results

The survey approach resulted in 79 government respondents, 379 private veterinary clinics and 14 wildlife rescue organisations being invited to complete the survey electronically. Thirty-seven survey responses were collected during the submission period. Thirteen responses contained information that could be used, reporting 340 individual cases of CPT in macropods; the remainder were empty responses that contained no information. The survey responses were provided from a variety of sources including government bodies ( $n = 8$ ), wildlife rescuers ( $n = 10$ ) and members of the public ( $n = 6$ ); not all participants disclosed their type of organisation. Twelve respondents made direct contact instead of completing the survey, resulting in an additional 12 individual cases being reported. The Melbourne Veterinary School and Wildlife Victoria (wildlife rescue organisation) provided a further 28 cases and 543 cases respectively in addition to the survey and direct contact cases, making up a total number of 923 individual cases. Species reported as affected were western grey kangaroo ( $n = 1$ ), eastern grey kangaroo ( $n = 601$ ) and swamp wallaby (*Wallabia bicolor*) ( $n = 5$ ).

Spatial data were available for the majority of reported cases ( $n = 918$ ), covering 36 LGAs across Victoria (Figure 1). Whittlesea (Chi-square goodness of fit test, z-score = 4.96,  $P < 0.01$ ) and Hume (z-score = 2.56,  $P < 0.01$ ) had a significantly higher number of cases per 1000 km<sup>2</sup> than the other LGAs. The three CPT classification accuracy tiers differed significantly in the number of cases



**Figure 1.** Distribution of all macropod chronic phalaris toxicity cases (number of cases per 1000 km<sup>2</sup>) in macropods from all tiers combined (n = 918), from 2011 to 2021 in Victoria. Cases are categorised by local government area (LGA): AL, Alpine; BA, Banyule; BC, Bass Coast; BL, Ballarat; CA, Cardinia; CG, Central Goldfields; CO, Colac Otway; CS, Casey; GB, Greater Bendigo; GG, Greater Geelong; GL, Glenelg; GS, Greater Shepparton; HE, Hepburn; HU, Hume; LA, Latrobe; MA, Mount Alexander; MB, Moorabool; ME, Melton; MI, Mitchell; MN, Manningham; MO, Moira; MP, Mornington Peninsula; MR, Macedon Ranges; MS, Mansfield; MU, Murrindindi; NG, Northern Grampians; NI, Nillumbik; SB, Strathbogie; SG, South Gippsland; SP, Southern Grampians; TO, Towong; WA, Wangaratta; WE, Wellington; WH, Whittlesea; WO, Wodonga; YR, Yarra Ranges. LGAs in grey did not have any cases.

(Table S2), with the least accurate tier 3 having more cases than tier 2 and similarly, tier 2 having more than tier 1 (Chi-square goodness-of-fit test,  $P < 0.01$ ). Amongst the LGAs, the City of Hume ( $z$ -score = 2.39,  $P < 0.01$ ) had a higher than average case number for Tier 1 (n = 19) (Figure S1). The City of Whittlesea ( $z$ -score = 2.88,  $P < 0.01$ ) had a higher case number for tier 2 (n = 288) (Figure S2), and both City of Hume ( $z$ -score = 2.54,  $P < 0.01$ ) and Whittlesea ( $z$ -score = 4.98,  $P < 0.01$ ) had a higher case number for tier 3 (n = 611) per unit area (Figure S3).

The total CPT case count per LGA correlated positively with both the abundance of *P. aquatica* ( $R^2 = 0.115$ , coefficient = 0.066, SE = 0.024,  $t = 2.702$ ,  $P < 0.01$ ) and the abundance of kangaroos ( $R^2 = 0.218$ , coefficient = 0.002, SE =  $5.47e^{-4}$ ,  $t = 0.95$ ,  $P < 0.01$ ) (Figure 2). This was consistent across tiers for the abundance of *P. aquatica* (Tier 1  $P = 0.02$ , Tier 2 and Tier 3  $P < 0.01$ ) and the abundance of kangaroos ( $P < 0.01$  for all three tiers) (Table S3).

The number of CPT cases associated with temporal data was 639 (Figure 3) (Tier 1 [n = 19], Tier 2 [n = 76] and Tier 3 [n = 544]). July had a significantly higher case count than other months, and this was consistent across all tier levels (total cases  $z$ -score = 2.58,  $P < 0.01$ ; Tier 1  $z$ -score = 2.94,  $P < 0.01$ ; Tier 2  $z$ -score = 2.24,  $P = 0.01$ ; Tier 3  $z$ -score = 2.54,  $P < 0.01$ ).

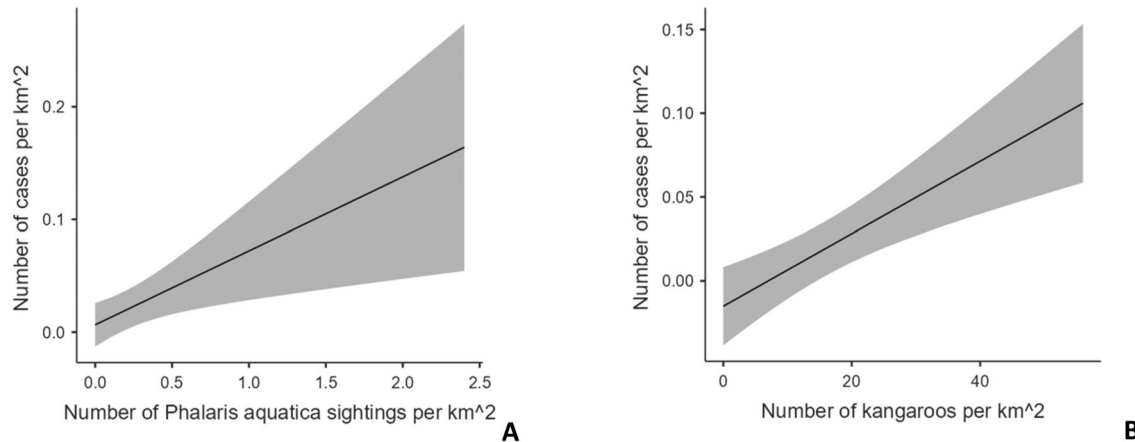
There was a significant negative correlation between the number of cases in a year and the rainfall of that year in March ( $R^2 = 5.22e^{-2}$ ,

coefficient =  $-0.158$ , SE = 0.0646,  $t = -2.45$ ,  $P = 0.016$ ) and April ( $R^2 = 0.0484$ , coefficient =  $-0.0777$ , SE = 0.033,  $t = -2.35$ ,  $P = 0.02$ ) of each specific LGA, but no correlation was found for May rainfall ( $R^2 = 5.76e^{-4}$ , coefficient = 0.0132, SE = 0.0526,  $t = 0.251$ ,  $P = 0.803$ ) (Figure 4). March and April rainfall were also negatively correlated with cases for Tier 3 (March  $P = 0.027$ , April  $P = 0.024$ ), and March rainfall for Tier 2 cases ( $P = 0.028$ ), but there was no significant correlation for Tier 1 (Table S4).

## Discussion

This retrospective study suggests that CPT is a widespread disease in macropods across the state of Victoria, Australia, with areas of high prevalence concentrated in the LGAs located centrally just north of state capital of Melbourne. The disease also has a strong seasonal pattern of occurrence in winter, peaking in July, with the magnitude of outbreaks negatively associated with the preceding rainfall in March and April. The hot spots for disease occurrence revealed in this study provide land and wildlife managers, wildlife rescuers and researchers with foundational knowledge on the areas to prioritise when managing or researching the disease. The correlations between disease occurrence and ecological factors explored expose new knowledge gaps for which future research is warranted.

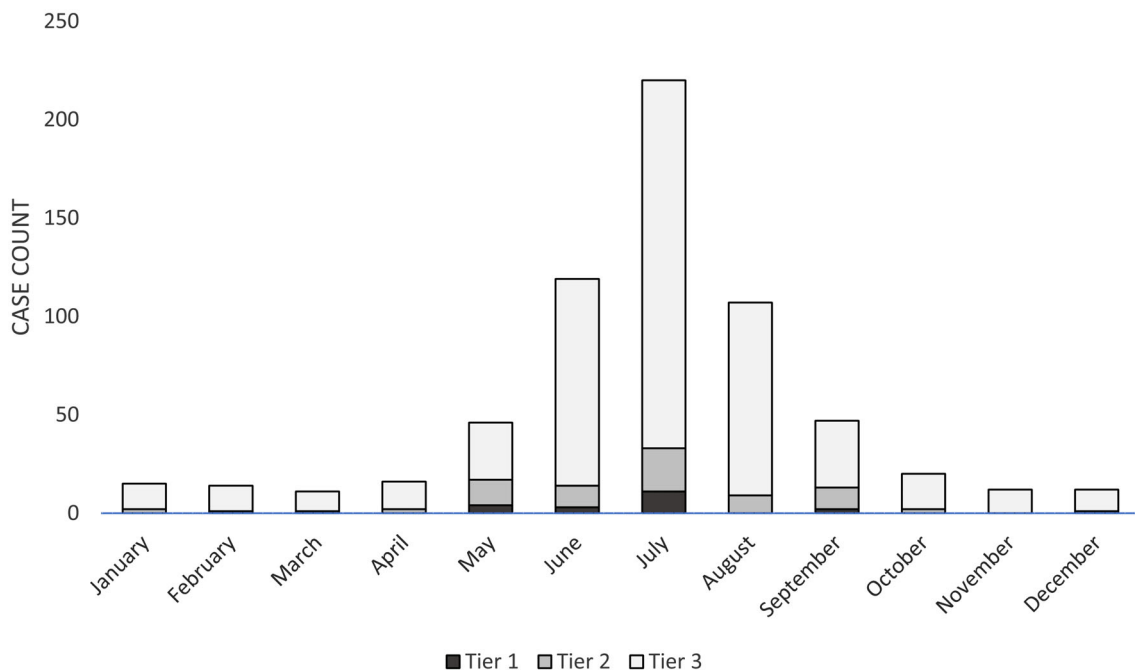
The positive correlation between case numbers, local abundance of *P. aquatica* and of grey kangaroos (*M. giganteus* and *M. fuliginosus*)



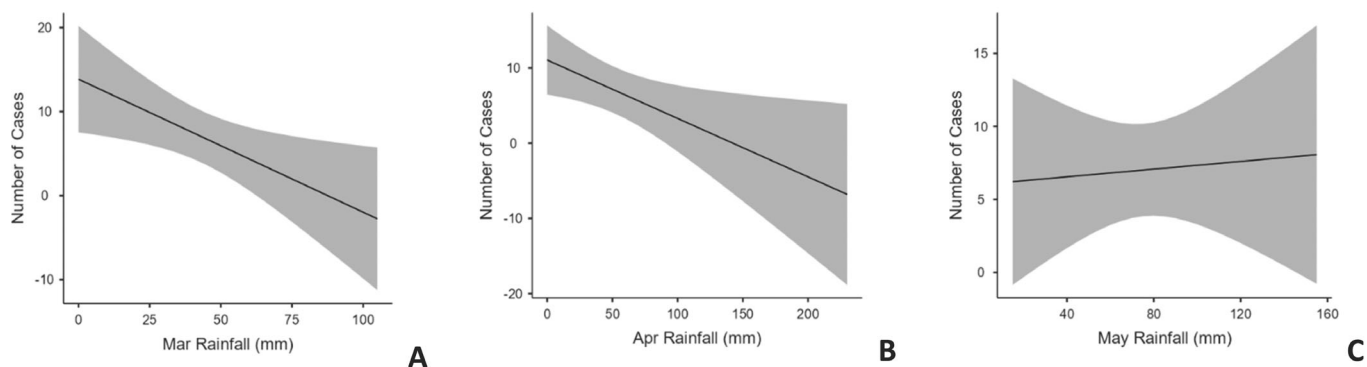
**Figure 2.** Linear regression of all macropod chronic phalaris toxicity cases (standardised to cases per km<sup>2</sup>) from 2011 to 2021 in Victoria versus (A) abundance of *Phalaris aquatica* ( $P = 0.009$ ) and (B) abundance of kangaroos (including *Macropus giganteus* and *Macropus fuliginosus*) ( $P < 0.01$ ). The line is the estimated marginal mean. The grey area represents 95% confidence interval.

was not surprising, given both factors are necessary causes of CPT, and an increase in either would presumably cause an increase in observed cases. However, it is important to note the geographical distribution of disease is likely to also be affected by a multitude of other factors such as soil type and anthropogenic disturbances to the environment. Low soil cobalt caused by limestone and sandstone substrate, high soil manganese, pH, and nitrogen have been known to increase the prevalence of CPT in sheep.<sup>10,24</sup> Anthropogenic changes to the environment such as increased urban developments can alter food availability for animals,<sup>25</sup> potentially resulting in abnormally high intake of *P. aquatica* if there are limited alternative feeds. One example of this is Plenty

Gorge Parklands ( $-37.631967, 145.105877$ ), Bundoora, Victoria, Australia, where within the past decade, urban development has effectively surrounded the area, landlocking the local macropod population within the park, which has large areas covered with *P. aquatica* due to historical agricultural use. This parkland has one of the highest reported case counts of CPT in the recent years. Importantly, factors such as remoteness of the area and low human population density could affect the detectability of cases, making it difficult to understand drivers of the disease. Diligent surveillance is required not only in areas where both the plant and animals are present in abundance, but also where additional factors such as urbanisation, remoteness of location or low



**Figure 3.** Total number of macropod chronic phalaris toxicity cases from 2011 to 2021 in Victoria. Data were separated into Tier 1 ( $n = 19$ ), Tier 2 ( $n = 76$ ) and Tier 3 ( $n = 544$ ) based on confidence of diagnosis with Tier 1 being the highest level of confidence.



**Figure 4.** Linear regression of all macropod chronic phalaris toxicity cases from 2011 to 2021 in Victoria from 2011 to 2022 versus the average monthly rainfall in (A) March ( $P = 0.016$ ), (B) April ( $P = 0.02$ ) and (C) May ( $P = 0.803$ ), for the respective LGA in the year of case occurrence. The grey area represents the 95% confidence interval.

human population density could increase the risk of disease or compromise the detection of cases respectively.

Low average rainfall in early autumn (March and April) was found to be a seasonal predictor for high numbers of cases in the subsequent winter. This finding agrees with the hypothesis that moisture stress after the first autumn rains and during the initial growth period of *P. aquatica* after sprouting can increase the level of alkaloid toxins in the plants.<sup>23,26,27</sup> Anecdotal evidence from wildlife rescue groups and government staff (Parks Victoria: K Moran personal communication, 2021) involved in the management of parks, further support this hypothesis. They reported a noticeable increase in the severity and number of kangaroos affected by CPT in 2018 and 2019, at known hot spots and in new regions, when rainfall in Victoria was recorded to be the lowest since 2006.<sup>28</sup> The extent of disease occurrence associated with autumn rainfall has opened the possibility of early predictions on the likely magnitude of outbreaks of CPT in future years, which may help land and wildlife managers be better prepared ahead of time.

The quality and detail of the survey responses varied greatly, leading to the creation of different tiers based on the accuracy of the diagnosis of CPT. Most cases fell into the tiers with less detail and specificity (Tier 2 and 3). Tier 1 cases reflected the highest level of diagnostic specificity; however, the relatively small sample size resulted in less statistical power for this tier. On the other hand, whilst having increased misclassification and recollection bias,<sup>29</sup> the two lower tiers were better at showing statistical relationships due to their higher sample sizes. A key challenge to obtaining a Tier 1 diagnosis is the need to avoid euthanasia via headshot to preserve the brain, which requires cooperation between land managers, veterinary professionals and wildlife rescuers. Unfortunately, such operations require a level of human and time resources that can be difficult to achieve in a field or outbreak setting. Consequently, most Tier 2 and 3 cases were reported by wildlife rescue groups where staff are experienced in wildlife handling and rescues but are not trained to provide clinical descriptions of animal health.

The need to work with data that are high in sensitivity but low in specificity, such as the Tier 3 data in the current study, highlights a major difficulty faced by investigators of wildlife diseases. The rapid

detection and accurate diagnosis of wildlife diseases are often very difficult, if not impossible, to achieve, and this may compromise data analysis that could shape the knowledge of disease processes. Several factors may contribute to this, including the absence or limited coordination and partnering of government-led collaborative surveillance networks of case detection, investigation and diagnosis, lack of response protocols and limited financial resourcing. Routine gold standard diagnosis is difficult to achieve when rapid response is required to maintain high animal welfare outcomes, especially if diagnosis requires specialised euthanasia of the animal as in the case of CPT for the preservation of diagnostic samples. The shortcomings in resources, combined with physical limitations, such as remoteness or difficult terrain, could mean that managers and researchers must learn to adapt surveillance strategies to encompass a certain degree of bias and uncertainty by utilising data that are of lesser quality.

This study demonstrates how the quality of data collected during disease surveillance and research programs can be improved by collecting data at different specificity levels. This may become useful in outbreak settings where there are insufficient human resources to perform a gold standard diagnosis for every affected animal. Strategic utilisation of limited resources can produce much greater effects if used thoughtfully. Tier 2 data, for example, can inform on the magnitude of an outbreak whilst still maintaining some specificity based on clinical observations. Diagnostic accuracy could be further enhanced if at least one representative case during each outbreak was diagnosed at tier one standard, confirming the presence of disease. In addition, the accuracy of Tier 3 data could be improved by standardising the collection and validation of information for each case. For nonveterinary organisations, such as animal rescue organisations, government or private individuals, the most important information to collect is the reason for rescue, date, location and species of the animal. The reason for rescue should be reviewed by the individual or organisation after the incident to confirm it is consistent with the description of the actual scenario based on structured reports from staff who attended the animal. For a more complete record, a description of the animal's age or life stage, sex and the animal's condition (e.g. broken right leg, wobbly gait) and the fate of the animal (e.g. euthanasia, relocated and rehabilitated) should also be recorded. For incidents where multiple animals are showing

similar conditions, the number of affected animals should be recorded with each animal having their own individual case record, and an estimate of the number of nonaffected animals in the group should also be noted. For veterinary organisations or organisations that can access veterinary professionals, a diagnosis should be obtained in addition to the information stated above, with the method of diagnosis (e.g. PCR, postmortem and clinical assessment) stated. Anyone working with wildlife should adopt a high standard of record keeping because these data are critical to disease surveillance and management. It would also be beneficial for large organisations, such as universities or government sectors involved in wildlife disease and management, to frequently communicate with the private sector to share their knowledge and help improve data collection, with the possibility of shared databases between organisations.

Like the CPT data, the external datasets obtained to explore predictor variables, including rainfall, the abundance of kangaroos and the abundance of *P. aquatica*, had different levels of detail affecting the accuracy of results and limiting confidence in inferences drawn. Out of the three datasets, the abundance of *P. aquatica* was the least accurate, because it was simply a count of the total number of verified sightings of the species in each local government region. There was no information on the bioavailability of the plant, which would require detailed estimation using metrics such as cover-abundance. In comparison, the rainfall data and the abundance of kangaroo data are more accurate, as they are estimates based on local weather station records and ground and aerial surveys, respectively. However, due to the large areas of certain LGAs, some with large range in elevation and diverse vegetation communities and topological features, the local climate and kangaroo abundance at the exact locations of disease occurrence may have varied from the overall average for the region. This challenge cannot be mitigated unless CPT case, kangaroo population distribution and environmental data become available at a finer level. This is difficult because the current surveillance efforts do not allow more detailed and consistent recordings of large number of cases. Further analysis based on collection of data from more structured and systematic surveillance or toxicological experiments will be required to further support our findings regarding the relationship between CPT and biological and environmental variables.

The results from this study provide a strong epidemiological foundation for better management of macropod welfare in response to the emerging wildlife disease, CPT. The findings aid in identification of critical locations and time points at high risk of disease, allowing managers to plan for and implement intervention and management strategies. The study also demonstrated the strengths and weaknesses of utilising wildlife records of differing levels of quality from private organisations and members of the public in disease surveillance. Although the quality of most data collected is currently lower than that of gold standard investigations, this source of knowledge has great potential to complement the current monitoring methods adopted by governments and universities and can cover significantly larger areas. It is important for wildlife disease personnel to acknowledge the current limitations in disease surveillance and the value of disease reporting from organisations of varying levels of scientific knowledge, because the combined information will create a more efficient and sensitive monitoring system.

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## Conflicts of interest

The author declares no conflicts of interest.

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**Data S1.** Supporting Information.

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