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Wombats and domestic livestock as potential vectors of *Cryptosporidium* and *Giardia* in an agricultural riparian area

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Abstract. Emerging pathogenic diseases are a significant burden on global economies and public health administrators. In Australia, the pathogens *Giardia* and *Cryptosporidium* are widespread in riparian areas subject to urban or agricultural contamination. Bare-nosed wombats (*Vombatus ursinus*) occur at relatively high densities alongside domestic cattle in agricultural riparian areas in south-eastern Australia and may transmit protozoan pathogens. We assessed the distribution of wombat scats and cattle pats on streambanks and screened them for the presence of *Giardia* and *Cryptosporidium*. The density of wombat scats declined with increasing distance from water while the cover of cattle pats increased with distance from water, but only at sites subjected to low cattle usage. We were unable to find any *Cryptosporidium* species or genotypes known to infect humans in either wombat or cattle faeces. One cattle sample contained *Cryptosporidium bovis*, a cattle-specific organism unlikely to be zoonotic. *Giardia duodenalis* (Assemblage E), a non-zoonotic pathogen, was detected in four cattle samples, but no wombat samples tested positive for *Giardia*. Our results suggest that while wombats represent a low-pathogen risk there is a need for consistent monitoring of potentially harmful waterborne and chlorine-resistant *Giardia* and *Cryptosporidium* pathogens in drinking-water catchments.

Additional keywords: cattle, drinking water, pathogen, zoonoses.

Introduction

Infectious diseases caused by zoonotic pathogens can be transmitted between wildlife, domestic animals and humans and are a significant burden on public health professionals and therefore global economies (Daszak *et al*. 2000; Jones *et al*. 2008). The protozoan waterborne parasites *Giardia* and *Cryptosporidium* are widespread in riparian areas subject to urban or agricultural contamination (Thurman *et al*. 1998) and are known to infect humans, livestock and wildlife (Heitman *et al*. 2002). Contamination by both *Giardia* and *Cryptosporidium* in 1998 was reported in Sydney's (Australia) water supply system following long periods of heavy rainfall and faecal contribution by livestock and wildlife was suspected (Cox *et al*. 1998; Clancy 2000).

Giardia species have been identified in the faecal matter of marsupials in parts of Australia (Thompson *et al*. 2008), and it is possible that the pathogens may also be present in the faecal matter of bare-nosed wombats (*Vombatus ursinus*). Wombats are large herbivorous burrowing mammals that occur in relatively high densities in riparian corridors adjacent to agricultural areas in south-eastern Australia (Skerratt *et al*. 2004). Wombats defaecate while moving and feeding (Lunney and O'Connell 1988), and make up to 10 deposits per night (B. Triggs, pers. comm.). Domestic cattle also use riparian zones because of the availability of water, shade, protection and forage (Powell *et al*. 2000). Adult cattle are known to defaecate up to 12 times per day, with typical daily mass of dry matter being ~4 kg (Marsh and Campling 1970). Faecal contamination by cattle is common near watercourses and has been recognised as a major water quality issue (Davies *et al*. 2004; Cox *et al*. 2005). Direct inputs into streams have been shown to be as high as 10% of total daily defaecations (Davies-Colley *et al*. 2002). Dairy cattle share a similar distribution to that of the wombat in eastern Australia. Zoonotic pathogens are common in dairy cattle, and the presence in cattle pats has been shown to be as high as 62% for *Cryptosporidium* and 89% for *Giardia* (Becher *et al*. 2004; Davies *et al*. 2005).

Given the close association between cattle and wombats across most of their range, we expected that wombats, as well as cattle, would be significant vectors of zoonotic pathogens. The high abundance of wombats in riparian zones in drinking-water supply catchments has raised suspicion about their possible role as vectors for zoonotic pathogens (Cox *et al*. 2005). Our study therefore aimed to (1) examine the use of riparian habitats by wombats (and cattle) by quantifying the locations of scats and pats

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in relation to the stream, and (2) objectively assess whether they are a source of the protozoa *Giardia* or *Cryptosporidium*.

Materials and methods

The study was conducted in Kangaroo Valley, New South Wales, Australia (34°43′S, 150°31′E) ~150 km south of Sydney (Fig. 1). Remnant patches of Eastern Riverine Forest, interspersed with invasive exotic plants, exist along many of the drainage lines in the study area. Currently, most floodplain and lower terrace areas are used for dairy farming. Riparian areas are often sown to exotic pastures and are heavily used by livestock for access to water. All waterways in the study area are also located in the Shoalhaven catchment, an important part of Sydney's drinking water supply.

Study sites

Sixteen sites were chosen, each comprising a 100-m section of stream bank, extending up the bank to the point where the slope was level. The distance from the water's edge to the edge of the bank ranged from 7 m for the steeper banks to 16 m for the gently sloping banks. Sites were separated by distances of 320–1000 m. The minimum distance between sites was based on the maximum distance travelled by radio-collared wombats (300 m) in an earlier study undertaken in Kangaroo Valley (Giles and Lonnon 1999).

Sites were categorised as low ($\leq$6 burrows per 100-m length of streambank) or high (>9 burrows $(100\ \text{m})^{-1}$) wombat use. The number of wombat burrows can be used as an index of wombat density (Rishworth *et al*. 1995). The use of paddocks with unrestricted streambank access by cattle was categorised as low (5.4 ± 4.1, mean $\pm$ s.e. cattle day^{-1} during the past 15 years) or high (79.3 ± 4.1 cattle day^{-1}) (Borchard *et al*. 2008). Usage by cattle was determined by the extent and size of cattle tracks, degree of faecal spoilage, trampling and overgrazing. Thus there were two factors, cattle usage (low, high) and wombat usage (low, high), with four replicates of each possible combination. The cattle found in the study area were part of large dairy herds.

Dung and scat distribution

At each of the 16 sites we assessed the density of wombat scats (scats m^{-2}) and the cover of cattle pats (%) within 1-m^2 contiguous quadrats along 10 transects placed perpendicular to the stream. Data were averaged over the 10 transects to obtain average values in relation to distance from the stream up to a maximum of 16 m. Regression analysis was used to examine relationships between cattle pat cover (and wombat scat density) in relation to distance from the stream for low- and high-cattle-usage sites separately (averaged over both levels of wombat usage).

Faecal sampling and collection

In August 2006, 55 wombat and 35 cattle faecal samples were collected opportunistically from dung and scat deposits at 11 of the 16 study sites and analysed for the presence of *Cryptosporidium* and *Giardia*. Only fresh material was used, and only one sample was collected from each deposit. Samples were refrigerated before analyses.

DNA extraction, PCR amplification and sequence analysis

Parasite DNA was extracted directly from faeces using the Mo Bio Powersoil DNA kit (Mo Bio Laboratories Inc.) and samples were screened for the presence of *Cryptosporidium* and *Giardia* by amplifying the 18S rRNA gene of the parasite using a two-step nested PCR. Amplified DNA fragments were separated by gel electrophoresis and purified using the Mo BIO Ultraclean15 Kit, according to the manufacturer's instructions. The purified PCR products were then sequenced using an ABI Prism™ Dye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA) according to the manufacturer's instructions with the exception that the annealing temperature was raised to 58°C

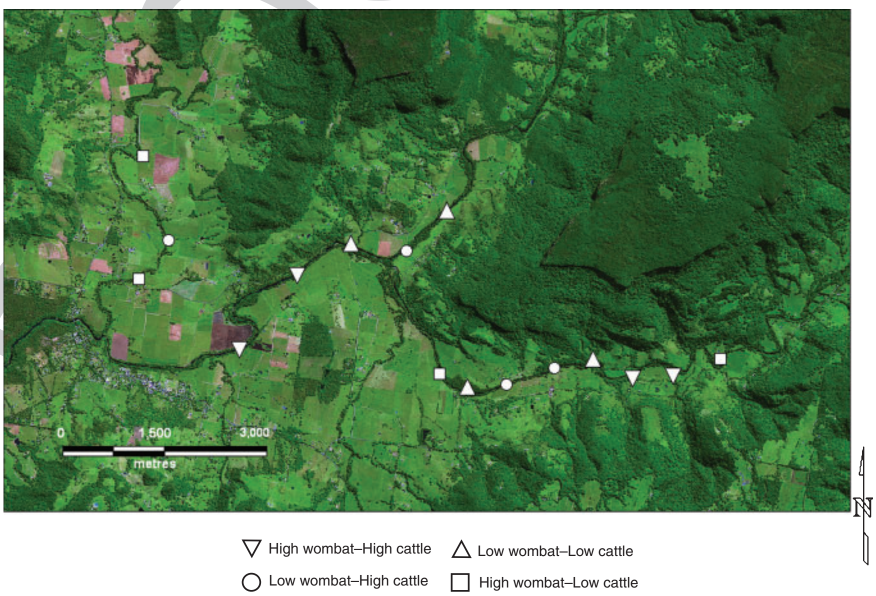

Fig. 1. The location of the Kangaroo Valley study area and approximate location of the 16 study sites (Mapinfo 2007).

for *Cryptosporidium* and 60°C for *Giardia*. Sequences were analysed using Chromas 2.3 (Technelysium Pty Ltd). Additional *Cryptosporidium* and *Giardia* sequences were obtained from GenBank and aligned using ClustalW (http://clustalw.genome.jp).

Statistical analyses

We used linear and non-linear models to explore potential relationships between the independent variable (distance from water) and the dependent variables (cattle pat and wombat scat densities), for high- and low-cattle-usage sites separately (Sigma Plot 2002 Vers. 8; SPSS Inc., Chicago, Illinois).

Results and discussion

None of the wombat scats and only a single cattle sample was positive for any *Cryptosporidium* spp. The positive cattle sample contained the non-zoonotic species *Cryptosporidium bovis*. Although *Giardia duodenalis* Assemblage E was detected in four of the cattle samples from three separate sites, no wombat samples were positive for *Giardia*. *C. bovis* has only recently been described, and has been identified only in cattle samples and is therefore considered unlikely to be zoonotic (Fayer *et al*. 2005). *G. duodenalis* (*syn. G. intestinalis* or *G. lamblia*) consists of seven genotypes (Assemblages A–G). Only assemblages A and B are zoonotic, while E is mainly found in livestock and not known to be infectious to humans (Lebbad *et al*. 2010).

The absence of human-infectious genotypes of either *Cryptosporidium* or *Giardia* in both wombat and cattle faeces suggests that these animals, at the time of sampling, represented a low risk to the drinking water supply in the catchment we investigated. The applicability of these results to other catchments in eastern Australia is largely unknown, but the results are consistent with studies showing that faeces of native animals represent a lower risk to water supplies than that of domestic or agricultural livestock (Heitman *et al*. 2002; Feng *et al*. 2007).

Dairy cattle, particularly young cattle, are considered a high-risk source of human-infective *Cryptosporidium* and *Giardia* (Farizawati *et al*. 2005). In our study area, tracks used by cattle in the riparian zone were frequently spoiled by cattle dung, consistent with studies elsewhere (Powell *et al*. 2000). As these tracks are narrow and commonly used by wombats, transfer of pathogens to wombats through ingestion of faecally contaminated plants is a strong possibility. Despite this, we found no evidence of any protozoan pathogens harmful to humans in the wombat scats, and no apparent protozoan transfer. Indeed, no clear relationships were apparent between cattle and wombat usage in the riparian zone, as density of wombat scats was largely independent of the cover of cattle pats ($P > 0.05$). We found that wombat scats were more abundant than cattle pats close to water, and the abundance declined with increasing distance from water ($F_{1,14} = 19.7$, $P = 0.0002$, $R^2 = 0.44$) (Fig. 2*a*) while the cover of cattle pats increased markedly with distance from water ($F_{1,14} = 33.4$, $P < 0.001$, $R^2 = 0.68$) (Fig. 2*b*). Both of these trends at the low-cattle-usage sites were not apparent at high-cattle-usage sites ($P > 0.15$).

Notwithstanding the fact that no zoonotic organisms were detected in our study, we recorded relatively heavy faecal deposition from both wombats and cattle within 16 m of the stream (Fig. 2*a, b*), well within the range of the riparian zone. The high faecal load of both species in the riparian zone probably increases the risk of transfer of potentially zoonotic organisms between wombats and cattle. The highest priority in riparian zones within water supply catchments would be to minimise faecal contamination from cattle, given that they are known vectors of *Cryptosporidium* and *Giardia* contamination (e.g. Becher *et al*. 2004; Davies *et al*. 2005). Prevention of water supply contamination should focus on efforts to exclude cattle from riparian zones, and by far the most effective method is through exclusion fencing. Our results suggest that although wombats generally defaecated closer to the stream than cattle, cattle faeces presented a greater risk to human health than did wombat faeces. Well vegetated riparian buffers from streambanks to upland areas may also reduce the risk of the export of pathogens into streams (Parkyn 2005).

Our data suggest that wombats are unlikely to be significant vectors of *Cryptosporidium* and *Giardia*, though seasonal differences in occurrence are possible, similar to the results of a study on eastern grey kangaroos (Power *et al*. 2005). Further studies of the sources of riparian-borne parasites are needed to ensure that water supplies are protected in sensitive areas such as drinking water catchments.

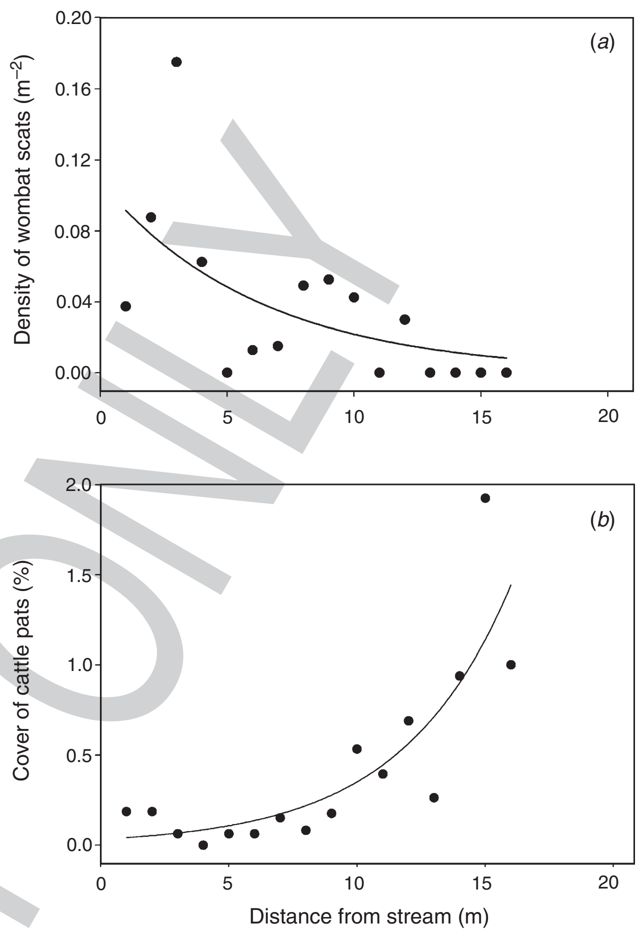

Fig. 2. (*a*) Density of wombat scats (m^{-2}) and (*b*) cover of cattle pats in relation to distance from the edge of the stream at sites of low cattle use.

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