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14	Ross River virus disease activity associated with naturally occurring non-tidal flood
15	events in Australia: A systematic review
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26 Abstract

Ross River virus (RRV) disease is the most common and widespread mosquito-borne disease 27 in Australia, resulting in considerable health and economic cost to communities. While 28 29 naturally occurring non-tidal flood events may enhance mosquito abundance, little is known about the impact of such events on RRV transmission. This paper critically reviews the 30 existing evidence for an association between naturally occurring non-tidal flood events and 31 32 RRV transmission. A systematic literature search was conducted on RRV transmission related to flooding and inundation from rain and riverine overflow. Overall, the evidence to 33 34 support a positive association between flooding and RRV outbreaks is largely circumstantial, with the literature mostly reporting only coincidental occurrence between the two. However, 35 for the Murray River, river flow and height (surrogates of flooding) were positively and 36 37 significantly associated with RRV transmission. The association between non-tidal flooding 38 and RRV transmission has not been studied comprehensively. More frequent flood events arising from climate change may result in increased outbreaks of RRV disease. 39 40 Understanding the link between flood events and RRV transmission is necessary if resources for mosquito spraying and public health warnings are to be utilized more effectively and 41 efficiently. 42 43 **Keywords** 44 45 Ross River virus, non-tidal, flood, outbreak, transmission

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Flood events around the world have had significant impact on the transmission of
mosquito-borne diseases (World Health Organisation 2013). In the past 20 years, outbreaks
of West Nile virus disease in the Czech Republic, Romania and Italy (Hubalek et al. 1999,
World Health Organisation 2013), malaria in the Dominican Republic, Costa Rica and
northern Peru, and the resurgence of dengue in the Americas (World Health Organisation
2013) have all been attributed to flooding.

A 'flood' is defined as: "a general and temporary condition of partial or complete 57 inundation of normally dry land areas from overflow of inland or tidal waters from the 58 59 unusual and rapid accumulation or runoff of surface waters from any source" (Geoscience Australia 2013). Thus flooding can arise from rain, riverine overflow, abnormally high tides 60 or higher than usual sea levels. Such events have the potential to spur a surge of mosquito 61 62 populations (Wilks et al. 2006) by stimulating egg-hatching (Liehne 1988) or by providing breeding opportunities for adult mosquitoes (Russell 1993). High numbers of mosquitoes thus 63 increase the opportunity for disease transmission (Liehne 1988). 64

In Australia, flooding associated with high tide occurs regularly, potentially 65 contributing to baseline arboviral activity in the locations where such inundation occurs. 66 However, non-tidal flood events arising from excessive rainfall and/or riverine overflow are 67 irregular events that have the potential to initiate arboviral activity over and above baseline 68 69 levels. These flood events prompt the mobilization of considerable post-flood management 70 resources, including public health responses to mitigate the risk of arboviral outbreaks -asignificant cost to the community. Therefore, this review focuses only on non-tidal flood 71 events arising from excessive rainfall and/or riverine overflow. 72

In Australia, naturally occurring non-tidal flood events have been linked to arboviral
outbreaks of Murray Valley encephalitis, Kunjin virus infection and Ross River virus (RRV)
disease (Wilks et al. 2006). Of all the mosquito-borne infections, RRV is the most common

76 and widespread in Australia. An annual average of more than 4,000 cases was recorded 77 between 2001 and 2013 (Communicable Diseases Australia 2014) with this figure probably underestimating the true infection rate because of "under-presentation and underuse of 78 79 laboratory testing in endemic areas" (Russell and Dwyer 2000, p.1694). RRV infection can result in considerable morbidity, including severe arthritis and chronic fatigue (Harley et al. 80 2001, Hickie et al. 2006). The annual cost of diagnosis, medical treatment and lost 81 productivity is conservatively estimated to be between \$4.1 and \$4.7 million (Woodruff and 82 Bambrick 2008). State and local governments incur indirect costs through mosquito control, 83 84 public health alerts and research. RRV disease therefore has a significant economic impact on Australian communities. 85

Understanding the relationship between flood events and RRV transmission can help minimize disease risk and make the best use of limited resources. If a positive association exists then defining the location and timing of mosquito population surges with greater precision can mean more timely health alerts and more targeted spraying programs, reducing the likelihood of insecticide resistance (Russell 1993, Weinstein 1997). Alternatively, if there is no association then local government and public health resources can be redirected to other flood mitigation activities.

The purpose of this paper is to review the existing evidence for an association between naturally occurring non-tidal flood events (hereafter referred to as 'flood events', 'flood' or 'flooding') and RRV disease activity, and to identify knowledge gaps and future research needs.

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Method

An initial literature search was performed using keywords and MeSH terms including
"Ross River" AND flood* OR inundat*. Search engines including EBSCOhost, ProQuest,

101 Web of Knowledge and Scirus were used to search databases Medline & Pubmed, Cinahl,

ScienceDirect, JSTOR, Scopus, Web of Science, Biological Abstracts, Current Contents and
E-Journals. Articles were also sourced from the citation lists of relevant articles identified in
the literature search. A Google Scholar search was also conducted to uncover gray literature.
The titles and abstracts of all publications yielded from the search were initially reviewed to
identify suitable articles; the full articles of which were reviewed.

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Inclusion and Exclusion Criteria.

Articles that referred to flooding, inundation, surface water and/or water-logging in relation to RRV outbreaks and transmission were included in the preliminary stage of the review. Articles that referred to flooding arising solely from higher than usual tides, a series of high tides or irrigation were excluded. Articles that referred to flooding arising from a combination of tidal inundation and rainfall were also excluded as the flooded areas were predominantly those normally inundated by tidal waters.

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Results

A total of 118 articles were identified, with 92 articles sourced from the electronic literature search, 25 articles from the reference lists of these publications and one 'gray' article from the Google Scholar search. Among these articles, 22 were identified as relevant and included in this review (Table 1). Of these 22 articles, 20 reported on 15 outbreaks associated with flooding and two statistically analyzed the relationship between flooding and RRV transmission.

123 RRV outbreaks following flooding were identified in four main regions across the
124 continent – northeast Australia (Townsville and the Atherton Tableland), northern central
125 Australia (Katherine), southeast Australia (New South Wales, Victoria and South Australia)

and southwest Western Australia, with the largest number of outbreaks occurring in thesoutheast corner of the continent.

The southeast corner is geographically dominated by the Murray-Darling River system 128 129 which drains freshwater from southern Queensland and the western side of the Great Dividing Range in New South Wales (NSW) into the Southern Ocean via South Australia 130 (SA). This review found that flood-related outbreaks in the southeast corner largely arose 131 around this river system, in particular the Murray River that forms the border between NSW 132 and Victoria. Flood-related outbreaks around the Murray-Darling River system resulting 133 134 from high rainfall occurred in 1956 (Anderson and French 1957), 1979-80 (Mudge 1980), 1983-84 (Boughton et al. 1984, Hawkes et al. 1985, Liehne 1988), 1990-91 (Marcon 1991) 135 and 1992-93 (Selden and Cameron 1996). An outbreak in 1976 (Mudge 1977) also followed 136 137 excessive rainfall and widespread flooding (Kelly-Hope et al. 2004); however, in this instance, the outbreak occurred six months after the flood and therefore may not have been 138 linked unless groundwater persisted throughout the summer. Although there was no 139 140 indication that groundwater persisted, mosquito numbers were high at the time of the outbreak (Mudge 1977) indicating that groundwater was at least available for vector 141 breeding, but the origin of that groundwater is not clear. 142

In 1971 (Seglenieks and Moore 1974) and 1993 (Norris 1993, Dhileepan 1996), flood-143 related outbreaks occurred around the Murray River at times when local rainfall was scarce. 144 145 High rainfall further upstream was thought to contribute to the higher-than-average river levels, at least for the 1993 flood. Therefore, not all RRV outbreaks around the Murray River 146 were determined by local rainfall and some may be initiated by riverine overflow. Indeed, Bi 147 148 et al. (2009) used monthly flow of the Murray River in SA as a surrogate measure of flooding and found that it was positively and statistically significantly associated with monthly RRV 149 cases at a lag of one and two months. Williams et al. (2009) also found that RRV incidence 150

increased on average 177/100,000 (95% Confidence Interval: 145-209/100,000) with each
meter increase in height of the Murray River in SA, after adjusting for mosquito abundance.
Interestingly, for the Williams et al. study, increased rainfall was not a predictor at all and
therefore could not be considered a confounder. However, it was noted that the height of the
river had been regulated via irrigation waters and therefore surface water due to irrigation
may account for RRV activity rather than river height. It is not known if human activity also
impacted on the results of the Bi et al. (2009) study.

In 1886, Natimuk Creek in mid-western Victoria flowed in January for the first time in 158 159 16 years after heavy rainfall (Wolstenholme 1993). Flooding of the creek preceded a disease outbreak characterized by symptoms consistent with RRV. The local physician assumed the 160 outbreak was dengue fever; however, Wolstenholme argues that Ae. aegypti, the main vector 161 162 of dengue, had never been collected in Victoria at that time, and that the dispersal of dengue had occurred along railways that had not yet reached the outbreak location. Therefore, 163 Wolstenholme suggests that the outbreak, preceded by riverine flooding, was likely due to 164 RRV rather than dengue. 165

In the northeast of the continent, outbreaks occurred in the summer of 1945 on the
Atherton Tableland (Dowling 1946) and in Townsville in 1998 (Kelly-Hope et al. 2004).
Both outbreaks were preceded by heavy rainfall and the formation of swamps and
floodwaters (Kelly-Hope et al. 2004). While the pathogen responsible for the 1945 outbreak
was not confirmed, it was assumed to be RRV since the symptoms of the patients were
consistent with RRV disease.

In northern central Australia, the Katherine River overflowed its banks in late January
173 1998 after very heavy rainfall, flooding the township of Katherine (Whelan 1998). Although
174 RRV cases in February were around 80% lower than the previous February, RRV cases in
175 March were twice that of the previous year. It was suggested that cases did not present until

176	March because mosquito spraying during February reduced RRV transmission and/or
177	because the flood interfered with access to medical services, delaying diagnosis.
178	In southwest Western Australia (WA), an outbreak in 1989 followed flooding arising
179	from high summer rainfall (Mackenzie et al. 1994). However, further information about the
180	timing or location of the flood in relation to the outbreak was not uncovered and no
181	information about case numbers or incidence was provided.
182	
183	Discussion
184	Quality of Evidence for Flood-induced RRV Transmission.
185	The evidence to suggest that a positive association exists between naturally occurring
186	non-tidal flooding and RRV transmission is largely circumstantial. Articles that address
187	outbreaks simply report that non-tidal flooding was observed prior to, or around the time of,
188	the outbreaks. We found only two studies that quantified the association between flooding
189	and RRV transmission (Bi et al. 2009, Williams et al. 2009). These studies used flow and
190	height of the Murray River as surrogates for flooding and demonstrated a significant and
191	positive association with RRV transmission. However, neither study actually provided
192	evidence that flooding had occurred and the results of the Williams et al. study may have
193	been confounded by the local presence of irrigation. Consequently, any association between
194	RRV transmission and flooding has not yet been demonstrated by robust evidence.
195	
196	How Flooding May Impact on RRV Transmission and Outbreaks.
197	RRV transmission is determined by a complex interaction between vectors, hosts and
198	humans. Flooding may impact on all these factors thereby influencing the transmission of
199	RRV.
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Flooding and Vectors.

Vector abundance is an important determinant of RRV transmission (Russell 1993, 202 2002) and flooding may promote RRV transmission by promoting vector abundance. High 203 204 mosquito numbers have been observed following flooding in southwest WA (Mackenzie et al. 1994), along the Murray River in SA (Seglenieks and Moore 1974, Mudge 1977, Mudge 205 et al. 1980, Woodruff et al. 2002), in the Murray Valley of Victoria (Russell 1986a, 1994, 206 207 Dhileepan 1996) and western NSW (Boughton et al. 1984, Hawkes et al. 1985). Flood events may promote vector abundance by extending predator-free breeding habitat, increasing 208 209 mosquito egg hatching, increasing the number of species contributing to the mosquito pool and extending the period over which mosquitoes are abundant. 210 In Australia, the two main RRV vectors are the Aedes and Culex mosquitoes (Russell 211 212 1993). Culex annulirostris (Skuse), the dominant inland mosquito, is a major contributor to baseline mosquito populations each year (Harley et al. 2001, Russell 2002) by making use of 213 the margins of permanent water sources for egg laying (Russell 1986b, 1987). However, this 214 species is more likely to survive in temporary pools than in more permanent bodies of water. 215 The adult mosquitoes prefer shallow pools for breeding while the immatures develop faster 216 and have lower mortality, probably because temporary pools are warmer and have fewer 217 predators than more established water bodies such as lakes (McDonald 1979, McDonald and 218 219 Buchanan 1981, Mottram and Kettle 1997). Therefore, flood events may be ideal for 220 providing temporary pools for mosquito breeding, thereby promoting the abundance of Cx. annulirostris over and above the usual numbers that exist each year. Indeed, in the 221 Kimberley (WA) Cx. annulirostris mosquitoes were significantly more abundant during flood 222 223 years than non-flood years (t = 2.21; df = 12; P < 0.05; using data of Broom et al. 2003). Flood events may also provide opportunities for immigrant vector species to increase in 224

number. Over a period of years, the salt marsh coastal species *Ae. camptorhynchus*

226 (Thomson) and Ae. vigilax (Skuse) have been encroaching on inland areas of southwest WA and southeast Queensland, respectively (Lindsay et al. 2007, Biggs and Mottram 2008). 227 Dryland salinity in these locations has resulted in new brackish water habitats that these 228 229 species have been able to exploit. Both species are vectors of RRV and indeed in 2004, this same area of Queensland recorded a high incidence of RRV disease believed to be related to 230 dryland salinity (Biggs and Mottram 2008). Thus, flood events in areas of dryland salinity 231 232 may promote virus transmission by extending salt marsh habitat for coastal vector species that have already migrated into inland areas. 233

234 Flood events promote egg hatching of the Ae. floodwater species [e.g. Ae. bancroftianus (Edwards), normanensis (Taylor), sagax (Skuse), theobaldi (Taylor) and 235 vittiger (Skuse)], thereby substantially contributing to the mosquito pool. The eggs of these 236 237 species withstand desiccation over long periods, possibly years, and therefore largely remain 238 dormant during non-flood years but hatch after flooding and extensive rain, when they become a significant pest (Russell 1986a, 1993, 1994). These species are particularly 239 prevalent after flooding in the southeast of the continent (Russell 1993). In western NSW, 240 widespread flooding following heavy rainfall generated large numbers of floodwater species 241 that preceded the first cases of the 1983-84 outbreak (Boughton et al. 1984, Hawkes et al. 242 1985, Liehne 1988, Russell 1994). Mosquito numbers were particularly high where the 243 244 incidence of the disease was highest (Hawkes et al. 1985, Russell 1986a). In the northern 245 reaches of the continent, Ae. normanensis also produces drought-resistant eggs that hatch after flood events (Russell 1994). Like Cx. annulirostris, it too exploits temporary ground 246 pools for breeding (Whelan and van den Hurk 2003). Therefore, flooding may promote 247 248 vector abundance by stimulating the emergence of a variety of mosquito species in addition to promoting favorable breeding habitat. 249

250 Flooding may also extend the period over which mosquitoes are normally abundant thereby extending the RRV transmission period. In southern Australia, the Ae. floodwater 251 species are typically abundant in late spring and early summer, while Cx. annulirostris is not 252 253 abundant until late summer and autumn (Russell et al. 1991, Russell 1993) when mean ambient temperatures exceed 17.5°C (Russell 1986b). The floodwater species also undergo 254 'instalment hatching', where egg batches hatch approximately every two weeks until the egg 255 bank is exhausted (Bader and Williams 2011). Flood-induced instalment hatching could 256 provide a means by which mosquito abundance remains consistently high throughout spring 257 258 and summer before Cx. annulirostris dominates in late summer and autumn. The emergence of two main vector types over sequential time periods means that the RRV transmission 259 period is potentially extended. Indeed, where Culex and Aedes species coexist in coastal 260 261 locations (Russell 1988, Ryan et al. 2000, Whelan and van den Hurk 2003, Hu et al. 2006) 262 the virus is more likely to persist (Glass 2005), improving the opportunity for viral transmission. 263

264 A number of the Ae. species, in particular the floodwater species, may be capable of transmitting the virus soon after emerging as adults, even before taking a blood meal. RRV 265 isolates have been obtained from newly emerged adult Ae. females in the field (Broom et al. 266 1989, Russell et al. 1992), from field-collected larvae (Dhileepan et al. 1996) and from the 267 268 ovaries of laboratory-inoculated females (Kay, 1982) suggesting that the virus is transmitted 269 vertically from one generation to the next (Russell 2002). For several locations in WA, significant increases in RRV notifications occurred within weeks after favorable 270 meteorological conditions (Lindsay et al. 1993a, b); a lag time too short for adult mosquitoes 271 272 to rely on viremic hosts as a source of infection. Several authors (Kay 1982, Lindsay et al. 1993a, Dhileepan et al. 1996, Russell 2002) suggest that vertical transmission may underlie 273 the persistence of the virus in nature. If that is the case, flooding may have the potential to 274

trigger rapid onset of seasonal RRV transmission by stimulating the emergence of
'transmission-ready' *Ae*. adults that may have otherwise remained dormant. Indeed, several
outbreaks have occurred soon after the formation of rain-induced groundwater in Queensland
in 1945 (Dowling 1946), SA in 1979 where indigenous mammals were absent "in any
numbers" (Mudge et al. 1980, p.627), NSW in 1983 (Boughton et al. 1984, Hawkes et al.
1985, Russell 1986a, b) and WA in 1989 (Lindsay et al. 1993b, Mackenzie et al. 1994).

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Not all flood events provide suitable conditions for vector breeding. A moderate flood 282 283 around the Murray River in Victoria in 1973 resulted in high numbers of mosquitoes while a flood of *unprecedented* height produced numbers that were only 3% of the previous year 284 (McDonald 1979). Floodwaters may be unsuitable for vector breeding because of water 285 286 depth, surface movement and lack of vegetation (McDonald 1979). In the coastal wetlands of 287 Darwin in the Northern Territory (NT), flooding from seasonal rainfall reduces Ae. vigilax populations by rendering the sites unviable for oviposition (Kurucz et al. 2009). Flooding 288 289 can also flush out larvae or possibly promote mosquito predation by increasing fish and aquatic insects (McMichael et al. 2006, Jacups et al. 2009). Only once floodwaters have 290 291 receded are remnant pools thought to be suitable enough for mosquito breeding (McDonald 1979, Russell 1998, Brown and Murray 2013). Around the Murray River, mosquito species 292 293 do not recommence breeding until temporary ponds stabilise and vegetation starts to develop 294 (McDonald 1979) and in the inland districts of southwest WA, mosquito larvae density does not increase until temporary water bodies start to decrease in surface area (Carver et al. 295 2009). Virus transmission may also be associated with the flow rate of floodwaters. For 296 297 West Nile virus disease, slow moving bodies of water increase the odds of infection while moderate moving water bodies protect against infection (Nolan et al. 2012). The slope of the 298 land is likely to affect the rate at which floodwaters move, with flat and steep terrain resulting 299

in slow and fast moving water respectively, and therefore creating or destroying mosquito
habitat respectively (Russell 1998). Therefore the impact of flood events on mosquito
abundance is likely to vary dependent upon the characteristics of the flood and the
topography of the flood-affected area.

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305 *Flooding and Hosts.*

RRV disease activity is not only driven by vector abundance. Indeed, when *Cx. annulirostris* populations are reaching peak abundance in the Top End (NT), RRV infections
are declining (Whelan et al. 1997). Clearly, factors other than vector abundance influence the
transmission of RRV. Vertebrate hosts play an important role in the transmission cycle
(Harley et al. 2001) and flooding has the potential to significantly impact on vector-host
transmission.

The most competent vertebrate hosts for RRV are the large-footed marsupials or 312 macropods; that is kangaroos and wallabies (Russell 2002). The most common and widely 313 314 distributed macropods are the eastern and western gray kangaroos (Dawson 2012). Their young predominately emerge from the pouch in spring and summer (Poole 1983) without 315 316 immunity to the virus (Kay and Aaskov 1989) and at a time when human RRV notifications are also starting to increase (Communicable Diseases Australia 2014). Flood events have the 317 318 potential to impact on vector-host transmission in two main ways; 1) enhancing host 319 susceptibility and 2) promoting the contact rate between host and vector. Flood events can result in a shortage of resources, physiologically stressing the animal and reducing its ability 320 to respond immunologically to pathogens (Carver et al. 2009). Flood events can also force 321 322 hosts to cluster on small parcels of dry land (Wilson 1957, Wilks et al. 2006, Jacups et al. 2008), providing an opportunity for vectors to contact many hosts in a small area. If newly 323 emerging adult floodwater mosquitoes are already infected through vertical transmission 324

(Russell 2002) and if a large proportion of the clustering hosts are non-immune juveniles,
then even a small number of infectious mosquitoes could infect many animals. The resultant
viremic hosts would then provide an opportunity for subsequent generations of mosquitoes to
become infectious. This explosion in infectious mosquito populations from enhanced vectorhost transmission may at least partially explain the outbreaks that follow some floods.

The common mouse has also been implicated as a possible host for RRV in northwest 330 331 Victoria (Carver et al. 2008), coastal NSW (Kelly-Hope et al. 2004) and the Murray Valley, SA (Anderson and French 1957). Carver et al. (2008) argue that the fecundity of the 332 333 common mouse can very quickly result in a high number of susceptible hosts providing a ready means of virus amplification. If flooding enhances food supplies then large numbers of 334 mice may be available, enhancing vector-host transmission. Indeed, flooding of the Murray 335 336 Valley in 1956 was followed by a higher than usual number of mice at the time of an RRV 337 outbreak (Anderson and French 1957). However, no other studies have reported high mouse abundance associated with flooding and RRV outbreaks, and therefore no conclusions can be 338 339 drawn on the basis of one study.

Floodwaters may also force animal hosts onto drier land that is closer to residential 340 areas. This closer proximity to animal hosts may be another means by which the 341 transmission of the virus to humans is enhanced under flood conditions (Jacups et al. 2008). 342 343 Frequent flood events, however, may reduce mosquito infectivity by increasing host 344 immunity. Glass (2005) found that the persistence of the virus, at least in Ae. vigilax, appears to be negatively associated with flood frequency and suggests that frequent flooding may 345 result in hosts being infected more frequently, increasing their immunity and reducing 346 347 viremia. Thus, more frequent flooding may reduce virus transmission and disease incidence. No evidence, however, is currently available to support or refute this suggestion. 348

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Flooding and Humans.

Flood events may also increase the risk of contact between humans and vectors, 351 increasing the risk of RRV transmission and outbreaks. During and after major flood events 352 353 the emergency response may result in large numbers of relief workers coming in close contact with floodwaters and therefore also mosquito habitat. Residents displaced from their 354 homes and accommodated in nearby evacuation centers that lack insect screens and other 355 356 mosquito protection measures may also be at risk of exposure to infectious mosquitoes. Flood events may also interfere with the conduct of routine mosquito control programs resulting in 357 358 greater mosquito abundance.

The risk of disease outbreak is also likely to be dependent upon the level of immunity 359 within the population (Harley et al. 2001). One of the largest RRV outbreaks occurred in 360 361 non-endemic regions of the South Pacific in 1979-80 (Aaskov et al. 1981a), where population 362 immunity was likely to have been low. In Australia, flood and heavy rainfall years are typically separated by lengthy periods of moderate rainfall and drought (Bureau of 363 364 Meteorology 2014a), providing an opportunity for immunity in the human population to wane. Although RRV infection is generally considered to confer lifelong immunity 365 (Horwood and Bi 2005), if new non-immune residents are added to the population, the 366 potential for an outbreak is high the next time flooding stimulates abundant infectious 367 368 mosquitoes. Transient agricultural workers and flood relief workers are considered to be 369 particularly susceptible to acquiring the virus because they are unlikely to have immunity compared to long-time local residents (Jardine et al. 2004, Brown and Murray 2013). 370 Alternatively, if viremic humans move into an area to assist with flood mitigation then 371 372 humans themselves may contribute to the vector-host transmission cycle as may have been the case in the South Pacific (Aaskov et al. 1981a), in Perth, WA (Lindsay et al. 1992) and 373 374 around the Murray River, SA (Seglenieks and Moore 1974). In addition, as previously noted, floodwaters may force both humans and animal hosts onto drier land, bringing them intoclose proximity (Jacups et al. 2008).

Flood events may also reduce the opportunity for humans to acquire the disease. 377 Where residents are required to relocate from flood-affected areas, the risk of contact with 378 infectious mosquitoes is likely to be reduced. For those residents that remain nearby to 379 floodwaters, the disruption to medical services may delay or prevent residents from seeking 380 medical attention (Brown and Murray 2013), thereby delaying or preventing diagnosis and 381 reducing the number of cases notified to public health authorities, as may have been the case 382 383 following the Katherine flood in the NT (Whelan 1998). In addition, an outbreak may also be avoided if the emergency response includes timely mosquito spraying, as may have also been 384 the case following the Katherine flood. 385

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Flooding and the Seasonality of RRV Transmission.

The seasonal timing of a flood event is likely to have an important impact on disease 388 transmission. In southern Australia, flood events occur at any time of the year (Pittock et al. 389 2006), including winter. Indeed, the southern Murray River catchment area experiences more 390 391 flood events in winter than summer (Pittock et al. 2006). Although winter flood events can stimulate high numbers of potentially infective Ae. floodwater species (Russell 1986a), RRV 392 393 epidemics still predominantly occur in summer and autumn (Harley et al. 2001). The low 394 incidence of RRV during winter and early spring months may be because susceptible hosts at this time are insufficient to sustain the vector-host transmission cycle. While the Ae. species 395 may be instrumental in initiating a potential outbreak through vertical transmission (Russell, 396 397 2002), RRV disease activity is unlikely to be sustained without the appearance of Cx. annulirostris adults which are not sufficiently abundant until summer and autumn (Russell 398 399 1986b, Dhileepan 1996). However, if the ground pools of a winter flood persist through to

400 spring and summer, or are replenished by ensuing rainfall, then a winter flood may be capable of at least exacerbating spring and summer outbreaks, as may have been the case 401 around the Murray River in 1976 (Mudge 1977) and 1992-1993 (Selden and Cameron 1996). 402 403 In northern Australia, the peak period for flood events is the wet season (Pittock et al. 2006) which occurs between October and March (Bureau of Meteorology 2014b). Mosquito 404 numbers are also high at this time and RRV cases peak between December and March. 405 406 Interestingly, *Cx. annulirostris* numbers, however, do not peak until the early-mid dry season (Whelan et al. 1997, Jacups et al. 2008) at a time when disease activity is declining (Whelan 407 408 et al. 1997). The reason for this decline in disease activity is not known but may again be explained by a lack of viremic hosts. By the time the dry season arrives, the majority of 409 juvenile macropods may have had sufficient time outside the pouch to acquire infection and 410 411 develop immunity. If that is the case, post-wet season flood events may not be capable of 412 initiating RRV outbreaks.

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414

Flooding and Lack of Transmission.

As previously discussed, not all flood events will necessarily lead to increased rates of 415 RRV transmission. The difficulty of providing evidence that supports or refutes a 416 relationship between flooding and transmission is that the lack of RRV transmission 417 418 associated with flood events is not of general interest, and consequently not reported in the 419 scientific literature. However, supportive evidence may be sought from other arboviral diseases. For Murray Valley encephalitis virus (MVEV), Russell (1986a) noted that an 420 outbreak occurred in the summer of 1973-4 after spring flooding, but not after spring 421 422 flooding in 1974 and 1975. Similarly, Forbes (1978) noted that the inland rivers of Australia flooded on 19 occasions but MVEV outbreaks occurred only on seven of those occasions. In 423 the Kimberley, although MVEV is most active in years following heavy rainfall and 424

425 extensive flooding, Broom et al. (2003) could only find a partial correlation between these ecological conditions and transmission. Similarly for West Nile virus, although disease 426 outbreaks followed flood events in Europe (Hubalek et al. 1999, World Health Organisation 427 428 2013), there was no increase in human cases following flooding in southeastern Kansas in the United States, despite the detection of arboviral activity at the time of the flood (Harrison et 429 al. 2009). Clearly, not all flood events promote arboviral transmission. Firstly, disease 430 431 transmission may vary with primary hosts. For example, although RRV and MVEV share *Cx. annulirostris* as a vector, wading birds appear to be the primary host for MVEV (Russell 432 433 and Dwyer 2000) rather than macropods, and therefore the impact of flood events on MVEV activity could be very different to that on RRV activity. Secondly, as previously indicated, 434 ambient temperatures and the flow rate of floodwaters is likely to impact on arboviral 435 436 transmission by influencing larvae abundance and development. In northern Australia, 437 monsoonal weather patterns (Bureau of Meteorology 2014b) result in heavy rains and fast flowing floodwaters that may reduce arboviral transmission, despite the year round 438 persistence of the principal vector Cx. annulirostris (Russell 2002); while in southern 439 Australia, ambient temperatures during the cooler months are too low for larval development 440 (Russell 1986b). Therefore, despite optimal flood conditions, arboviral transmission may not 441 occur unless other supportive ecological and environmental conditions are in place. 442

443

444 Alternative Explanations.

Evidence suggests that outbreaks are associated more so with high rainfall than
flooding. Rainfall has been identified as the single most important risk factor for RRV
transmission (Kelly-Hope et al. 2004). Of 55 outbreaks between 1886 and 1998, 20
coincided with significant rain, 16 coincided with rain and flooding and only three coincided
with flood alone (the environmental conditions of the remainder were not stated) (Kelly-Hope

et al. 2004). Indeed, for the current review, 80% of the flood-associated outbreaks also
coincided with above average rainfall. However, if above average rainfall is responsible for
RRV transmission by providing groundwater for vector breeding, then inasmuch as river
height and river flow are considered surrogates of flooding, so too may excessive rainfall.
Thus, flooding, minor or otherwise, associated with higher than average rainfall, river flow or
river height, may be the main determinant of RRV transmission rather than rainfall per sé.

The potential relationship between flood events and RRV transmission may also be 456 confounded by human activity such as the use of irrigation and dam level control. In 457 458 southwest NSW and northwest Victoria, outbreaks in 1992-93 and 1997 were centered on areas where flooding arose from a combination of rainfall and irrigation, or rainfall and river 459 overflow from dam level control (Norris 1993, McDonnell et al. 1994, Russell 1994, 460 Dhileepan 1996, Woodruff et al. 2002). Therefore it is not known if the outbreaks were 461 attributable to naturally occurring floodwaters from heavy rainfall or from human-induced 462 463 flooding from irrigation or dam water release. Jardine et al. (2008) statistically analyzed the impact of irrigation on RRV notifications in southern inland WA but did not detect a 464 significant association. If irrigation is standard practice from year to year, then this practice 465 466 may only be contributing to baseline disease incidence. Therefore outbreaks in some rural areas may be more attributable to naturally-derived flooding that occurs in addition to the 467 468 usual irrigation. However, in some regularly irrigated locations, outbreaks occur in the absence of additional rain-induced or riverine-induced flooding, as was the case in 469 470 Coleambally (NSW) in 1970 (Byrne 1984) and the Kimberley (WA) in 2002-03 (Jardine et 471 al. 2004). In the Kimberley, Cx. annulirostris numbers in irrigated areas were more than five times that of non-irrigated areas (P < 0.001) indicating that the irrigated areas were the likely 472 source of disease transmission (Jardine et al. 2004). However, as these irrigation practices 473 474 occur annually, outbreaks may occur for other reasons such as an influx of non-immune

475 (Jardine et al. 2004) or indeed viremic (Seglenieks and Moore 1974) transient agricultural476 workers.

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Limitations of Previous Studies.

Some important limitations were noted during the current literature review. For the 15 RRV outbreaks: 1) no article provided a definition of 'outbreak' or 'epidemic'; 2) two did not indicate the number of cases; 3) almost 75% did not confirm an outbreak by comparing case numbers or incidence rates with previous years; 4) one outbreak could be dismissed as such because case numbers were fewer than the previous year and 5) almost 50% of articles provided no or only partial serological evidence. Therefore, more than 85% of the reviewed outbreaks could not be verified as actual outbreaks.

486 Additionally, for more than 50% of the outbreaks, symptomatology was not reported. The serological test used by many laboratories is designed only to detect RRV-specific 487 antibodies (IgM) which can persist for up to 48 months post-infection (Australasian 488 489 Association of Clinical Biochemistry 2011). Therefore cases should only be confirmed as recent cases if they have both positive serology and RRV-consistent symptoms (Inverness 490 491 Medical Innovations 2008); otherwise, positive serology alone could be explained by past infection. Therefore, case numbers reported in the articles of this review could be inaccurate, 492 493 making it difficult to reliably associate RRV transmission with flood events.

For the two modeling (transmission) studies, standard notification data were sourced
from public health authorities. Prior to 2013, the detection of RRV-specific IgM without
symptoms was sufficient for a case to be notified to public health authorities (Communicable
Diseases Australia 2012). Therefore, not all notifications used for the modeling studies may
have been representative of recent cases. Furthermore, up to 75% of acute RRV cases are
asymptomatic and go undetected and un-notified (Aaskov et al. 1981b). Therefore, given the

likelihood of both under-reporting and over-reporting of cases, notification data used for
modeling studies are unlikely to be accurate. In addition, the location of infection is most
often assumed to be the case's residence (Department of Health 2005) and not necessarily
where the infection was acquired.

Finally, only one modeling study used data split from the main dataset to validate the
model (Bi et al. 2009). There was no indication that the model used by Williams et al. (2009)
had been validated.

507

508 **Future Research Directions and Recommendations.**

A substantial gap exists in understanding the impact of naturally occurring non-tidal 509 flood events on vectors, hosts and RRV transmission. More frequent extreme weather events, 510 511 such as storms and flooding, arising from climate change (McMichael et al. 2006) are expected to impact on mosquito habitats (Wolstenholme 1992, Mackenzie et al. 1993, Russell 512 1998) and therefore also on RRV transmission (Mackenzie et al. 1993, Russell 1998, Russell 513 and Dwyer 2000, McMichael et al. 2006). The exact impact, however, is difficult to predict 514 as environmental conditions vary from one location to another and flood characteristics vary 515 from one event to another. Furthermore, the impact of flood events occurring in different 516 seasons is also likely to vary. As with other predictor variables of RRV transmission, the key 517 518 to understanding the distinct role of flood events in the transmission of RRV is likely to 519 depend on studying the relationship in each discrete location (Gatton et al. 2005). However, determining a link between flooding and RRV transmission is likely to be 520 difficult where flood events go unreported. One possible means by which to study the 521 522 relationship is to use river height data to determine when a river has overflowed its banks,

resulting in flooding, minor or otherwise. Data for residential proximity to waterways may

also be of value to determine if residents living close to flood-prone waterways are at greater

525	risk of RRV disease than those living further away. By pinpointing areas of the community										
526	at most risk of acquiring the disease, insecticides can be used more judiciously, minimizing										
527	the risk of mosquito resistance (Weinstein 1997). Furthermore, if flood events and RRV										
528	transmission are not associated then valuable public health resources can be redirected to										
529	other flood-mitigating activities.										
530	As a result of this review it is recommended that future studies address:										
531	1. identifying the locations where naturally occurring, non-tidal flood-related										
532	outbreaks occur most frequently, e.g. Murray River, southeast Australia;										
533	2. determining if a statistical relationship between non-tidal flood events and RRV										
534	outbreaks in these locations exists;										
535	3. pinpointing the areas of each location where disease incidence is highest;										
536	4. determining the optimal location and timing of implementing post-flood mosquito										
537	management programs to minimize disease transmission and maximize the efficient										
538	use of flood mitigation resources.										
539											
540	RRV outbreaks inflict considerable cost on communities by impacting on the										
541	individual, health services, tourism and the local economy (Woodruff and Bambrick 2008).										
542	Determining when and where a community is most vulnerable to arboviral transmission can										
543	assist resource-limited public health authorities to implement more efficient mosquito										
544	management initiatives thereby minimizing the human and economic burden of disease.										
545	Understanding the relationship between non-tidal flooding and RRV transmission may also										
546	be applicable to other vector-borne diseases affected by flooding such as Murray Valley										
547	encephalitis, West Nile virus disease, dengue and malaria.										
548											

Conclusions

550	The current review reveals that the relationship between naturally occurring non-tidal
551	flooding and RRV transmission has not yet been comprehensively studied. The evidence to
552	suggest an association between the two is largely circumstantial. Resources used for
553	mosquito spraying and public health warnings are limited and need to be implemented at a
554	time and location that can make the greatest impact on RRV transmission. Therefore,
555	understanding the link between RRV transmission and flooding is essential if those resources
556	are to be utilized more effectively and efficiently.
557	
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563	
564	Conflicts of Interest
565	Nil.
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814	Table 1. Ross River virus outbreaks and transmission associated with naturally occurring non-tidal
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Period of outbreak	Location	Reference	Study type	Key findings	Comments
• 1886 Mar.	Natimuk, mid-west Vic	Wolstenholme 1993	Descriptive	30 cases High rainfall in DecJan.: Natimuk Creek flooded in Jan.	Cases not serologically confirmed
∘ 1945 Feb.–April	Atherton Tableland, north Qld	Dowling 1946	Descriptive; Mosquito survey	95 cases Swamp formation due to high rainfall occurring two weeks prior to commencement of outbreak	Cases not serologically confirmed RRV-competent mosquito species present
° 1956 AprMay	Murray Valley, NSW, Vic & SA	Anderson & French 1957	Descriptive	Estimate of 1,000-2,000 cases Exceptionally wet summer with excessive flooding along Murray River; many cases had contact with the River; mosquito abundance appeared no higher than usual; higher than usual number of mice observed	Cases not serologically confirmed Only 36 cases medically examined No mosquito survey reported
° 1971 FebApril	Murray River, SA	Seglenieks & Moore 1974	Epidemiological	High river level from Oct. 1970-June 1971 with shallow flooding 115 cases medically examined and most had contact with River Coincided with influx of seasonal workers from Qld Higher than usual mosquito abundance observed	Not all cases serologically confirmed No mosquito survey reported Authors suggest that seasonal workers from Qld may be viremic host
° 1976 Mar.	Murray Riverland, SA	Mudge 1977 Kelly-Hope et al. 2004	Symptom survey Review	Widespread flooding in winter and spring Disease incidence 344/100,000 High mosquito numbers observed at time of epidemic Coincided with influx of seasonal workers from Qld	Total number of cases not stated Not all cases serologically confirmed Outbreak occurred several months after flood thus the two may not be related No mosquito survey reported
° 1979-80 NovFeb.	Murray Riverland & Eyre Peninsula, SA	Mudge et al. 1980	Epidemiological	Disease incidence 360/100,000 (21 cases serologically confirmed with 80% symptomatic) Most cases occurred away from waterways Abnormally high spring rainfall resulting in large areas of groundwater accompanied by high mosquito numbers	No mosquito survey reported
^o 1983-84 JanFeb. (60% cases) Cases reported from OctJune	West of the Great Dividing Range, NSW	Boughton et al. 1984 Hawkes et al. 1985 Russell 1986a,b	Serological survey Descriptive Mosquito survey	1,196 cases serologically confirmed Flooding rains occurred before and during outbreak High mosquito numbers observed at time of epidemic	Symptom survey conducted in only one location (Griffith) Largest outbreak ever recorded in NSW RRV-competent mosquito species present
 1989 Spring- Summer 	Southwest WA	Mackenzie et al. 1994	Descriptive; Review	High spring and summer rainfall accompanied by flooding and large numbers of RRV-competent mosquito species	Specific location of flood not stated Case numbers or incidence not stated No mosquito survey reported
° 1990-91 Summer Peak in late Jan.	State-wide, Vic	Marcon 1991	Epidemiological	443 cases serologically confirmed and symptomatic compared to 40 in previous year; location of disease acquisition recorded for >80% High rainfall NovJan. with flooding of Darling Basin	Timing of flood in relation to outbreak not stated
• 1993 JanJune with 64% of cases occurring JanFeb.	Southwest NSW	McDonnell et al. 1994	Epidemiological	Ten-fold increase in notifications compared to previous year Attack rates ranged 387-770/100,000 Heavy rains with flooding from OctDec., 1992; Jan. 1993 rainfall was average	Not all cases serologically confirmed and symptom status not reported Irrigation in same area may have confounded results No mosquito survey reported
° 1993 JanApril	Murray Valley, inland north & northwest Vic	Dhileepan 1996 Norris 1993 Russell 1994	Descriptive Descriptive Review	1,210 cases serologically confirmed which was more than 20 times that of previous and following years Location of infection confirmed for 73% cases Mosquito survey showed high abundance	Irrigation practices may have confounded results

Period of outbreak / transmission	Location	Reference	Study type	Key findings	Comments
° 1992 Oct1993 June	Murray Valley/Riverland, Eyre Peninsula, SA	Selden & Cameron 1996	Case survey	821 cases serologically confirmed and >85% symptomatic Large areas of surface ground water after above average rainfall from late winter to summer	Cases surveyed up to 36 months post- infection, thus patients may not have accurately recalled location No mosquito survey reported
∘ 1997 Jan.	Murray River, NSW	Woodruff et al. 2002	Descriptive for flood- associated outbreak (Main study uses 1991-1999 data for statistical model)	Murray River area flooded for more than 30 days in Oct. 1996 following high rainfall and reservoir release Rainfall over summer was below average but ground pools from flooding persisted High mosquito numbers observed despite low summer rainfall	Case numbers or incidence not stated Reservoir release and irrigation were possible confounders No mosquito survey reported
° 1998 Feb.	Townsville, Qld	Kelly-Hope et al. 2004	Descriptive; Review	343 serologically confirmed cases Widespread flooding after heavy rains DecJan.	Case numbers retrieved from Communicable Diseases Unit, Queensland Health and therefore outbreak based on unpublished data No mosquito survey reported
° 1998 JanApril	Katherine, NT	Whelan 1998	Descriptive	17 cases compared to 22 cases for the same time in the previous year; more cases presented in March than previous year River flooded town after heavy rainfall in late Jan. High density of mosquito larvae first half of Feb. <i>Cx. annulirostris</i> (Skuse) predominant species Cases presented in March and higher number than previous year	Case presentation may have been delayed due to mosquito spraying and disruption of medical services Vector monitoring and insecticide use may have limited case numbers
t 1992-2004	Riverland, SA	Bi et al. 2009	Time-series Poisson regression	Monthly river flow positively associated with monthly RRV cases at 1 month lag Model was better predictor when weather variables included	River flow used as a proxy for flooding Outbreaks occurred in 1993, 1997, 2000 & 2001 but co-occurrence of flooding not stated
t 1999-2006	Murray Valley & southeast SA	Williams et al. 2009	Stepwise multiple regression	Combined river height and vector data predicted RRV incidence in Mid- Murray local government area	Flooding as a result of high river levels was based on personal observation Model validation not stated

841 °Outbreak; ^r Transmission; NSW=New South Wales; NT=Northern Territory; Qld=Queensland; SA=South Australia; Vic=Victoria; WA=Western Australia