



Animals as potential reservoirs for dengue transmission: A systematic review

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ABSTRACT

Dengue is a rapidly spreading mosquito-borne flavivirus infection that is prevalent in tropical and sub-tropical regions. Humans are known to be the main reservoir host maintaining the epidemic cycles of dengue but it is unclear if dengue virus is also maintained in a similar enzootic cycle. The systematic review was conducted in accordance to Cochrane's PRISMA recommendations. A search was done on PubMed, EMBASE, Scopus and Cochrane Library. Key data on animal dengue positivity was extracted and classified according to animal type and diagnostic modes. Of the 3818 articles identified, 56 articles were used in this review. A total of 16,333 animals were tested, 1817 of which were positive for dengue virus by RT-PCR or serology. Dengue positivity was detected in bats (10.1%), non-human primates (27.3%), birds (11%), bovid (4.1%), dogs (1.6%), horses (5.1%), pigs (34.1%), rodents (3.5%), marsupials (13%) and other small animals (7.3%). While majority of dengue positivity via serology suggests potential enzootic transmission, but regular dengue virus spillback cannot be excluded. With the exception of bats, acute infection among animals is limited. Further investigation on animals is critically required to better understand their role as potential reservoir in dengue transmission.

1. Introduction

Dengue is the most rapidly spreading mosquito-borne viral disease in the last decade. Transmission is the most active in the tropical and subtropical regions of the world. At least 128 countries, including 36 previously dengue-free countries in temperate regions, with a total estimated population of 3.97 billion people are at risk of dengue outbreak [1]. Globally, the estimated number of dengue infections is 390 million per year. Among these infections, only 25% are symptomatic and at least 1% are severe cases, mainly in children [2]. The number of symptomatic dengue cases has increased more than twice in every new decade between 1990 and 2013. This has resulted in about 1.14 million disability-adjusted life-year and about 9221 dengue deaths per year [3]. The total global cost due to dengue illness is estimated to be US

\$8.9 billion per year [4].

Dengue infection is caused by single-stranded RNA virus of the genus *Flavivirus* and family *Flaviviridae*. There are four antigenically-related dengue serotypes (DENV 1, 2, 3 and 4) which are genetically diverse, with a number of genotypes and clades of each serotype that have been associated with dengue disease severity [5] as well as a number of severe dengue epidemics [6]. Dengue virus infection is usually self-limiting and mild, but can also result in severe diseases classified as dengue hemorrhagic fever/dengue shock syndrome [7] or severe dengue [8] based on World Health Organization guidelines. Prevention measures are usually focused on mosquitoes' population control as well as removal and reduction of mosquito's breeding grounds. These measures can be effective but only over a short period of time and it is expensive to sustain as a long-term national strategic intervention [9]. While recent

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trials of the release of wolbachia-infected mosquitoes had demonstrated the feasibility and efficacy to reduce the mosquito populations, more prospective investigation and surveillance are necessary to assess any long-term implications among the Wolbachia/mosquito/virus interactions [10]. The only approved dengue vaccine does offer some form of protection, but remains limited in efficacy [11] and in vaccine implementation among seronegatives [12]. Furthermore, treatment remains largely supportive with appropriate fluid management as there is still no effective antivirals against dengue despite decades of search.

Dengue virus transmission likely originated from sylvatic cycles maintained between susceptible non-human primates and *Aedes* mosquitoes in the forests of Asia [13]. Sylvatic transmission cycles have also been described in the forests of Africa, but no evidence indicates likewise in the Americas [14]. Spillover of sylvatic dengue virus can occur in both rural and urban areas as zones of emergence. Given the adaptability of sylvatic virus for the human host and documented past infections that resulted in severe clinical manifestations, this can potentially sustain the natural horizontal human to human transmission via *Aedes aegypti* and *Aedes albopictus* mosquitoes [15,16]. Humans are known to be the main reservoir host in maintaining urban epidemic cycles of dengue [17]. While animals are commonly thought to be the reservoir host for flaviviruses transmitted by *Aedes* mosquitoes such as Zika virus [18] and yellow fever virus (YFV), there is insufficient evidence suggesting that for urban dengue transmission. Notably, Zika virus was first discovered in a monkey in Uganda [19] while non-human primates in South America are well established as reservoirs of YFV given their high susceptibility to YFV infection [14,20]. As such, the aim of this study is to systematically evaluate the role of animals in dengue transmission.

2. Material and methods

2.1. Search strategy and selection criteria

This study was carried out following the recommendations of the Preferred Reporting Items for Systematic Review and Meta-Analyses (PRISMA) [21]. There were two phases for our search strategy. The first phase involved using all the identified relevant index terms and keywords to do an extensive search in four different databases – EMBASE, PubMed, Scopus and the Cochrane Library. The second phase involved searching for additional articles based on the references found in the selected articles from the first phase but were not found in the database search. The following keywords were used in the first phase of search: ‘dengue’, ‘dengue virus’, ‘dengue infection’, ‘DENV’, ‘animals’, ‘dogs’, ‘cats’, ‘pigs’, ‘horses’, ‘bats’, ‘monkey’, ‘swine’, ‘chicken’, ‘poultry’, ‘rodents’, ‘rats’, ‘primates’, ‘ducks’, ‘insects’ and ‘birds’. The search was done in late October 2019 with no restriction on time. Importation of references and removal of duplicate references were done using the bibliographical software package, Endnote version X7 (Thomas Reuters, New York, NY, USA). The inclusion criteria was any of the following types of peer-reviewed articles involving dengue diagnosis in animals through direct testing of DENV1/2/3/4 or flaviviruses: observational studies, seroprevalence studies, cross-sectional studies, case-control studies or reviews. The exclusion criterion was any of the following types of peer-reviewed articles: case reports, case series, clinical trials, non-randomized/randomized controlled trials, animal model/in-vivo studies, in-vitro/cell-based studies, experimental studies or vaccine-related studies. All titles, abstracts and selected full reports were screened independently by two authors based on the inclusion and exclusion criteria. Discrepancies were resolved by consensus.

2.2. Data extraction

The following variables of interest were extracted from the selected studies: animal type, country, location of study, study period, species of animal involved, biological samples collected, dengue positivity, dengue

serotypes and reported dengue outbreak. The data extracted were cross-checked by another author and discrepancies were resolved by consensus. Data was classified into three categories of animals – bats, non-human primates and other mammals, and two main diagnostic modes – serology or RT-PCR. Other animals include specified animals that were neither bats nor non-human primates, or unspecified animals in their respective studies. Serology includes diagnosis by enzyme-linked immunosorbent assay (ELISA), plaque reduction neutralization test (PRNT), hemagglutination inhibition (HI), complement fixation (CF), and antigen tests (NS1). Studies were also classified into either forest or urban/rural setting, which encompassed non-forest sites near human settlement. Studies that involved both settings due to sample collection at multiple sites had their results separated into each of the categories as much as the literature informs.

2.3. Data analysis

On several occasions, a single study used different number of animal samples in multiple diagnostic methods. For the most of this review including the meta-analysis, dengue prevalence for each animal type was pooled by including one platform of diagnostic method, either serology or RT-PCR, that involved the largest number of samples in each study. Since some NHP and bat studies utilized multiple methods, dengue prevalence by serology and RT-PCR was pooled respectively by including both the serologic method that used the biggest sample and RT-PCR if applicable. Meta-analysis was conducted using STATA 14.2, with the metaprop software command. Each animal type was analysed separately, stratified by diagnostic type whenever possible. Forest plots that display prevalence of dengue positivity with 95% confidence intervals (CI) were generated for graphical representations. I^2 statistic values were calculated to quantify degree of heterogeneity among studies, where values of 25–50% represented moderate heterogeneity and values of >50% large heterogeneity among studies.

3. Results

A total of 3811 articles were identified from the initial database search, of which 886 were duplicates and removed. The titles and abstracts of the remaining 2932 articles were screened for their relevance yielding the selection of 110 articles for full-text review. After the review of these 110 articles based upon our inclusion and exclusion criteria (Fig. 1), a final list of 56 articles were selected for comprehensive study. Data from 55 articles were utilized in this review – two [22,23] different papers were identified to have utilized an identical sample, resulting in our exclusion of one [23] in the tabulation of figures. The following animal types were involved the final selected studies – bats ($n = 18$ studies), non-human primates ($n = 22$), birds ($n = 9$), rodents ($n = 5$), dogs ($n = 2$), pigs ($n = 3$), bovid ($n = 6$), horses ($n = 4$) and marsupial ($n = 3$). A collective of other mammals and reptile – one study involved caimans – not included in the list above were also mentioned across 8 articles. Overall, 31 studies exclusively studied a single animal type – bats ($n = 13$ studies), non-human primates ($n = 18$), other animals ($n = 10$) including pigs ($n = 1$), rodents ($n = 1$), dogs ($n = 1$), horses ($n = 1$) and birds ($n = 6$).

3.1. Non-human primates

Between 1954 and 2017, there were 2509 non-human primates (NHP) originating from 20 different countries (Argentina, Brazil, Costa Rica, Democratic Republic of Congo, French Guiana, Gabon, India, Japan, Kenya, Malaysia, Nigeria, Panama, Philippines, Puerto Rico, Rwanda, Senegal, Sri Lanka, Thailand, Venezuela and Uganda) that were tested for evidence of dengue virus infection (Table 1, Fig. 2). The names of species involved in each study are detailed in Supplementary Table 2. Of all studies that tested for evidence of DENV infection in NHPs, fourteen study sites from which NHP samples were collected

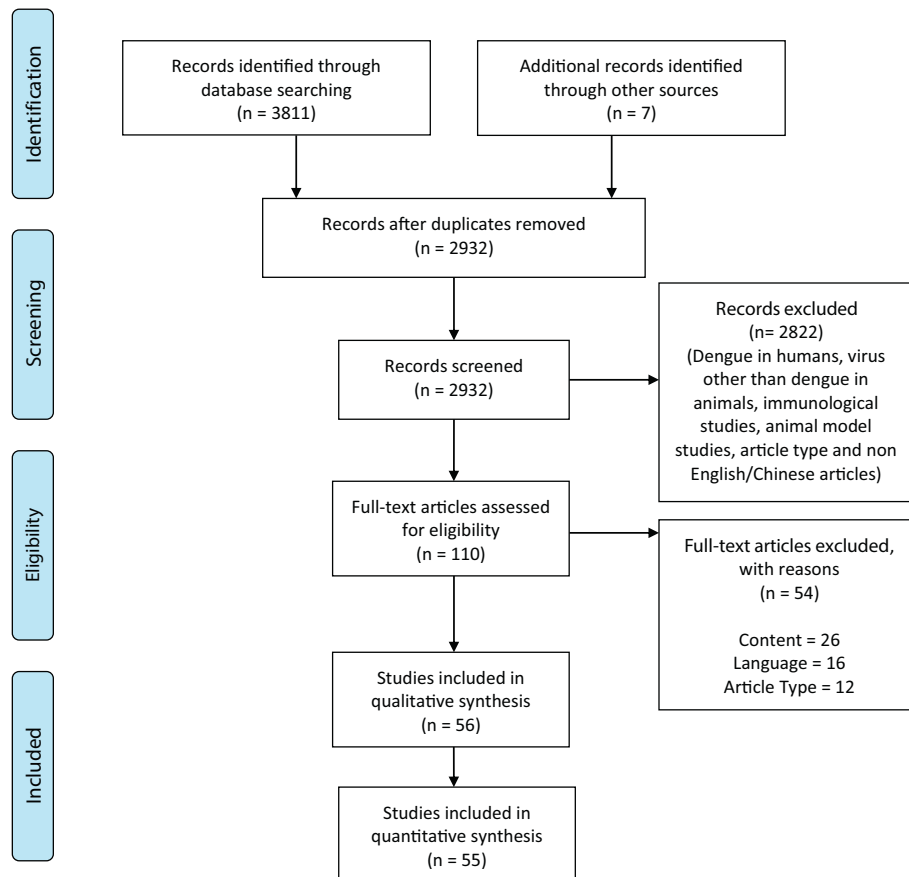


Fig. 1. PRISMA flow diagram.

reported dengue endemicity and past outbreaks (Table 2). Among NHPs (mainly monkeys) tested, 684 (27.3%) were found to be dengue positive, albeit mostly through serological tests (Fig. 3). All but one study reported the identification of anti-dengue antibodies through serological means. The remaining study identified the presence of antibodies against flaviviruses in general. Overall, there is a dearth in studies employing RT-PCR to detect dengue infection in non-human primates. Despite 13 of 22 studies were conducted after or examined samples dated after 2000, only 4 studies employed RT-PCR diagnostic to some extent. Only a single study utilized RT-PCR to identify dengue RNA genetic material in all their samples, identifying 8 positives of 155 (5.2%) monkeys tested. Our meta-analysis of studies based on one platform of detection method found a dengue prevalence of 24% (CI: 13–36%) in NHPs (Fig. 4). There was significant intra-group heterogeneity in the serology group (I^2 : 97.82%), and pooling of results from both serology and RT-PCR methods was not supported as given significant inter-group heterogeneity was noted ($p = 0.000$). There were 15 studies involving a forest setting [22,24–37], 12 of which were based solely in a forest. Of 7 studies involving the urban/rural setting [26,31,38–42], only 5 were exclusively based at such. Only one study involved the coastal setting [29]. Four studies constituted samples obtained from a mix of settings or from an unidentified source [43]. While most of the animals tested were dengue positive for serotype 2, it was observed that 8 of 18 studies with clearly published results distinguishing serotypes tested specifically for serotype 2 only (Table 1).

3.2. Bats

Between 1954 and 2018, a total of 2827 bats captured from twelve different countries were tested for evidence of dengue virus infection (Table 2, Fig. 2). The species names are detailed in Supplementary

Table 1. Bats originated from Australia, Brazil, China, Columbia, Costa Rica, Ecuador, French Guiana, Grenada, India, Mexico, Singapore and Trinidad. All except five studies involved sites associated with high dengue endemicity and occurrence of outbreaks (Table 2). All except seven studies used reverse transcription polymerase chain reaction (RT-PCR) as one of the or only detection method/s to identify the presence of dengue RNA genetic material. Of these studies, only one was conducted before the 21st century, indicating a paradigm shift in bat studies towards using RT-PCR only in recent years. Among the 1559 bats tested with RT-PCR, 90 (5.8%) bats were found to be dengue virus RNA positive. All, except four studies, used serological assay as one of the or only detection method/s to identify the presence of dengue antibodies. Among 1974 bats tested with serological assay, 266 (13.5%) bats were found to have antibodies against dengue virus. Based on including only one platform of detection method in each study, 10.08% of bats were positive, with 3.04% and 7.04% of dengue positivity observed from RT-PCR and serology assays respectively (Fig. 3). Overall, our meta-analysis of studies based on one platform of detection method found a 9% dengue prevalence (CI: 4–14%) in bats (Fig. 4). There was significant intra-group heterogeneity as I^2 exceeded 85% for both studies, but pooling of results from both serology and RT-PCR methods was supported as no inter-group heterogeneity was noted ($p = 0.309$). Among the urban/rural setting where bats were captured, there were 5.9% (65/1098) of bats positive for dengue virus RNA as compared to 5.6% (25/445) of bats captured in the forest setting ($p > 0.05$). Among the urban/rural setting where bats were captured, there were 13.1% (136/1036) of bats that were dengue positive by serology while no bats (0/91) captured in the forest setting was positive by serology ($p < 0.05$). There were 14 studies involving the urban/rural setting [44–57], 4 involving the forest setting [44,52,58,59]. Of which, 12 and 2 studies respectively were based in those settings exclusively. Only 1 study was of the coastal

Table 1
Study characteristics and dengue positivity among non-human primates.

Animal Types	Country	Location	Site Setting	Study Period	Dengue RT-PCR Positive Only (%); Serotype	Dengue Serology Positive Only (%); Serotype	Serological Test Type; Serotype Tested	Human Dengue Cases; Serotype	Dengue Serotype (Mosquitoes)	References
Monkeys	Philippines	Animal Breeding Facility	Urban/rural	2010	2/9 (22.2); D2.Asian Epidemic DENV (NS1 & E)*, D2.Asian Epidemic DENV (NS1)*	21/100 (21.0); D1,2,3	PRNT; Tested for D1/2/3/4	Remarkably high number of DENV cases was reported in 2010, more than triple the number reported in 2009 & Number of cases exceeded the Alert and Epidemic thresholds.		[40]
Monkeys	Asia	Imported Samples	Unknown	Before 1984		145/358 (40.5); D1,2,3,4	PRNT; Tested for D1/2/3/4			[43]
	India					0/33	PRNT; Tested for D1 only			
Monkeys	Japan Philippines	Luzon Island, Monkey Farm	Forest	1999		1/37 (2.7); D2 2/54 (3.7)	PRNT; Unspecified ELISA; Unspecified	35.2% and 16.9% of confirmed DENV and probable DENV cases in 770 patients		[28]
		(Monkeys originated from Zamboanga in Mindanao island and Tanay)								
Monkeys	Sri Lanka	Nature & Archaeological Reserve at Polonnaruwa	Forest	1986 1987 1995		2/16 (12) 41/44 (94) 52/244 (21); D2	ELISA; Unspecified ELISA; Unspecified ELISA; Tested for D2 only	1986–1987 epizootic		[33]
Monkey	Sri Lanka	Nature & Archaeological Reserve at Polonnaruwa	Forest	1987		64/68 (94); D2	PRNT; Tested for D2 only	All 4 serotypes isolated from humans before		[32]
Monkeys	Puerto Rico	Southwestern Region	Urban/rural	2010 & 2012		23/23 (100); D1, 3, 4	MNT; Tested for D1/2/3/4	Circulation of all 4 DENV types during 1992 to 2012.		[42]
Orang Utans	Malaysia	Sabah	Forest (Free Ranging)	1996–1998		11/40 (28)	IFA/ELISA; Unspecified			[23]
Orang Utans	Malaysia	Sabah	Forest (Semi-captive)	1996–1997		10/31 (32) 11/40 (28); D2 10/31 (32); D2	PRNT; Tested for D2 only	Denv-2 seroprevalence in native Bornean and migrants at 11/30 (37%) and 59/80 (74%) respectively. Humans sampled lived or worked on the boundaries of the Sepilok Forest Reserve.		[22]
Monkeys Galagos	Nigeria	Nupeko Forest	Forest	1969 (Lowland monkeys) 1971–1972 (Nupeko monkeys)		45/92 (48.9); D2 3/12 (25%); D2	PRNT; Tested for D2 only	486/1275 (38%) and 811/1816 (45%) seropositivity in humans by HI and PRNT respectively.		[25]

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Table 1 (continued)

Animal Types	Country	Location	Site Setting	Study Period	Dengue RT-PCR Positive Only (%); Serotype	Dengue Serology Positive Only (%); Serotype	Serological Test Type; Serotype Tested	Human Dengue Cases; Serotype	Dengue Serotype (Mosquitoes)	References
NHP	Kenya	Kwale & Kakamega County (Western & Coastal)	Forest	2014		14/34 (41%) (Coastal); 5/33 (15%) (Western); D1,2,3,4	ELISA; Tested for D1/2/3/4			[29]
Monkeys	Brazil	Atlantic Forest, Bahia, Northeast Brazil, municipalities of <i>Ilhéus</i> and Una	Forest (Free Ranging)	2006–2014		31/110 (28.2); D1,2,3,4	HI; Tested for D1/2/3/4	D1,2,3,4 are endemic to both municipalities with sporadic outbreaks		[36]
Monkeys	Brazil	Atlantic forest (Border of Pernambuco and Paraíba States) Zoo & Wildlife Screening and Recovery Center, Recife	Forest (Free Ranging)	2015–2016		14/49 (28.6); D1,2,3,4	PRNT; Tested for D1/2/3/4	D1, 2, 3 and 4 are endemic to the human population		[34]
NHP, Monkeys	Brazil	Zoos in Salvador, Brasília, Itapetinga National primate centre in Cabedelo, Salvador, Vitória da Conquista & Barreiras. Urban & Peri Urban sites; Lucena, Sapé & Santa Rita,	Urban/Rural	2012–2017	0/207 (Tested against Flavivirus)	2/6 (33.3); D1	PRNT; Tested for D1 only			[39]
NHP, Monkeys	Venezuela	Parque Zoológico Bararida, Barquisimeto, Parque Zoológico El Pinar, Caracas & Guri dick (8 free living)	Urban (Captive) & Forest (Free-ranging)	Before 2006		2/62 (3.2); D2	HI; Tested for D2 only	Dengue highly endemic in Venezuela since 1989.		[31]
NHP, Monkeys	Argentina	Unknown	Unknown	1993–1996, 2000–2012	8/155 (5.2); D2,3,4 (C/prM)	0/4	ELISA	Co-circulation of D1,2,3,4 since re-emergence in 1993.		[26]
NHP, Monkeys	Costa Rica	31 Lowlands locations	Forest & Urban/rural (Private land)	1993–1996, 2000–2012		53/209 (25.4) (Tested against flavivirus)				[26]
NHP, Monkeys	Malaysia	Unknown	Forest	1962–1964		139/221 (62.9); D2	PRNT; Tested for D2 only			[35]
NHP, Monkeys	French Guiana	Petit Saut Hydroelectric Dam	Forest	1994–1995		0/145	PRNT; Tested for D2 only	Dramatic increase in cases over the past 30 years, epidemic in 1991–1992 with up to 3000 cases.		[37]
Mandrill	Gabon	Lope National Park	Forest	1998–2006		2/25 (8); D2	PRNT; Tested for D2 only			[30]
NHP, Monkeys	Congo Basin; Uganda, Democratic Republic of Congo & Rwanda	Bwindi forest, Park National of the Volcanoes, Kinigi & Unknown locations	Forest	2001–2009		0/44				

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Table 1 (continued)

Animal Types	Country	Location	Site Setting	Study Period	Dengue RT-PCR Positive Only (%); Serotype	Dengue Serology Positive Only (%); Serotype	Serological Test Type; Serotype Tested	Human Dengue Cases; Serotype	Dengue Serotype (Mosquitoes)	References
NHP, Monkeys	Panama	Localities near the Canal zone (unknown setting)	Urban/rural	1954–1955		2/105 (1.9); D1,2	HI; Tested against D1/2 only (Only 2 positive samples further tested using PRNT)	Dengue epidemic in 1904, 1912, late 1941 to early 1942. At least one local outbreak occurred during 1934–1936. Sporadic cases observed in 1946. 57/213 (27%) residents of Caledonia (non-forested area) with D1/2 HI antibodies 195/892 (22%) residents of forested areas of Panama with D1/2 HI antibodies.		[41]
NHP, Monkeys	Senegal	Forest Gallery Near Ngari	Forest	2000		0/17; D2	ELISA; Tested for D2 only		D2 (<i>Aedes furcifer</i> , <i>A. taylori</i> , <i>A. luteocephalus</i> , <i>A. aegypti</i>)	[24]
NHP, Monkeys	Thailand	Chiang Mai (Captive Monkey) Chiang Rai (Captive Monkey) Maehongsorn (Captive Monkey)	Urban/rural	2008–2009		7/21 (33) 1/12 (8.3) 1/5 (20)	PRNT; Tested for D1/2/3/4 but results unspecified	6052 cases of DHF in 2008 & 2009. 1974 cases of DHF in 2008 & 2009. 942 cases of DHF in 2008 & 2009.		[38]
NHP, Monkeys	Argentina	San Cayetano, Corrientes Isla del Cerrito Isla Brasilera	Forest	2010	0/51 (Tested against flavivirus; NS5)	36/108 (33.3); D1,3	PRNT; Tested against D1/3 only	Indigenous circulation of DENV in Northern and Central Argentina since 1988.		[27]

* Phylogenetic analysis linked to human cases.

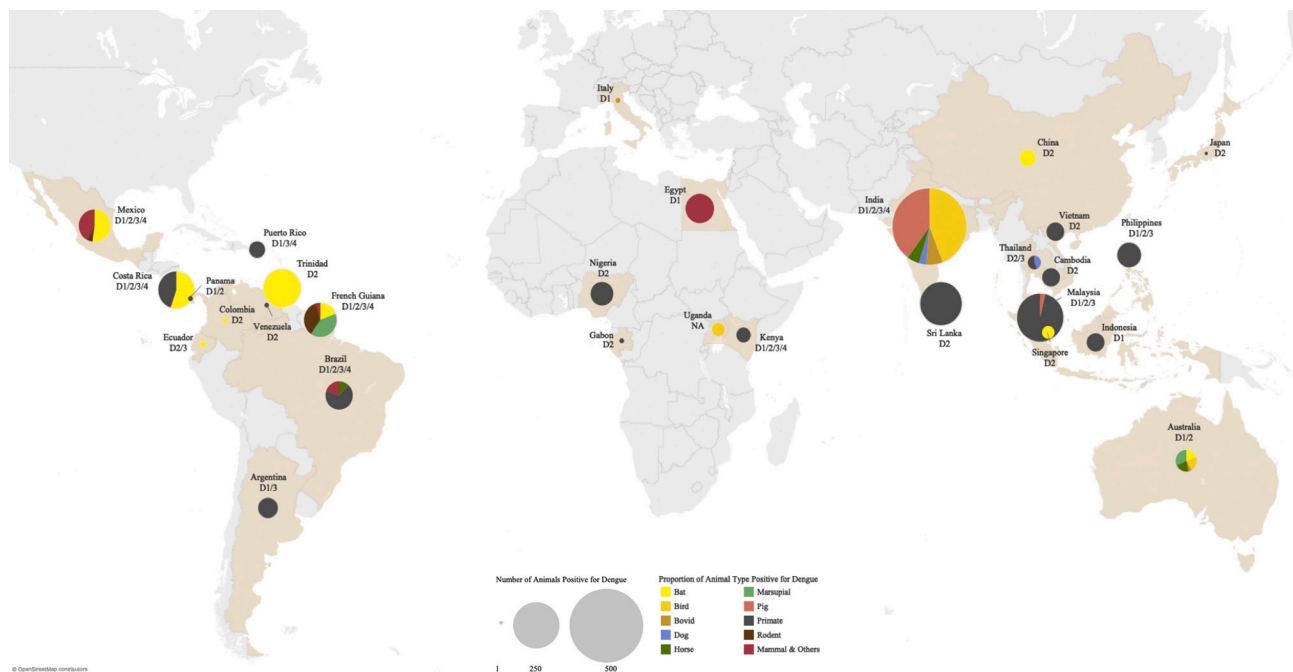


Fig. 2. Geographical distribution and proportion of dengue positivity reported among different animal types. NHP: Non-human primates; Others: Shrew, Sloths, Acouchy, Agouti, Porcupine, Armadillo and Kangaroo.

setting [60] and 1 was of unidentified origin [61]. The sole study conducted in the coastal setting reported 14.3% positivity for dengue by serology, comparable to the rate observed in the urban/rural setting. Most of the bats tested in Mexico and Costa Rica were reported to be infected with dengue serotype 2, even though dengue serotype 1, 3 and 4 were also detected. This observation may be associated to the predominant dengue serotype 2 circulation in the human community during the same period of time (Table 2).

3.3. Other animals

Between 1954 and 2016, there were 1406 dogs, 2083 bovid (e.g. sheep, cattle, goat), 1244 horses, 2218 birds (including hens, ducks & geese), 608 pigs, 1187 rodents, 31 elephants and a collective of small animals reported from 17 different countries (Australia, Brazil, Central African Republic, Chad, Czechoslovakia, Democratic Republic of Congo, Egypt, French Guiana, Gabon, India, Italy, Malaysia, Mexico, South Pacific Islands, Thailand, Uganda and Zambia) that were tested against dengue (Table 3, Fig. 2). Eleven of 24 studies involved sites associated with dengue endemicity and outbreaks. Names of the species are detailed in Supplementary Tables 3–11. Among the animals tested, 11.0% of birds, 4.1% of bovid, 1.6% of dogs, 5.1% of horses, 34.1% of pigs, 3.5% of rodent, 13.0% of marsupials and 7.3% of other small animals were found to be dengue positive (Fig. 3). All except three studies employed serological testing to identify the presence of antibodies against dengue - hemagglutination inhibition (HI; 12 studies), plague reduction neutralization test (PRNT; 5 studies), enzyme-linked immunosorbent assay (ELISA) screening followed by PRNT (2 studies) and mouse protection test (1 study). Majority of the studies on pigs and birds had utilized HI instead of PRNT, the gold standard for serological testing. Among the dogs tested with RT-PCR test, only 0.5% were found to be positive. Of 1187 rodents tested, 33 were positive by RT-PCR while 8 were positive by serology. Most of the positive rats (36/41) were from the forest. Meta-analysis of all animal types displayed high heterogeneity (Supplementary Table 14). Overall, most of the dengue positive animals were reportedly from the urban and rural areas [35,36,44,48,56,62–74]. Otherwise, six studies involved the forest setting [30,36,37,44,59,75], one was in the coastal setting [60], one was

unknown [76]. Notably, most of the animals tested were dengue positive for serotype 2.

4. Discussion

Dengue transmission is well-known to involve sylvatic (enzootic) cycle and urban endemic cycle which involve non-human primates in sylvatic habitat and humans in urban setting as reservoir hosts [17], respectively. As demonstrated in our study, a number of reports suggest other animals may play a role in both the sylvatic and urban endemic cycles as potential secondary hosts. Moreover, with increasing deforestation due to globalization and urbanization, animal and human populations are increasingly staying closer together with higher frequency of potential contacts [16,77]. It is not clear if dengue transmission can evolve to involve an enzootic cycle in an urban setting like the West Nile virus transmission among birds and/or a rural epizootic cycle that involves amplification of dengue virus within domestic animals. As a result, these may increase the risk of spillover and outbreaks affecting the human population in the rural and urban settings.

This systematic review summarized evidence that dengue virus can infect at least 84 species of 23 animal types, including 28 species of non-human primates (Supplementary Tables 1–11). On the contrary, West Nile Virus (WNV), another flavivirus, has also been shown to infect at least 225 species of birds and at least 29 animals (including horses, cattle, llamas, alligators, cats, dogs, wolves and sheep [78,79]). Animal species in both the Old and the New World have antibodies to WNV, but virus is rarely isolated from animals in the Old World compared with those in the New World. Unfortunately, this phenomenon is not clear for dengue virus as most studies were performed only with serological tests but not RT-PCR. Japanese Encephalitis virus (JEV) similarly infects an unusually wide range of animals, including equines, birds, dogs, bats and snakes [80], but they are reported to be dead-end hosts that are unable to infect mosquitoes. Pigs and birds are the major amplifying hosts of JEV, although infection usually does not produce clinical disease.

This systematic review showed that NHPs (27.3%), pigs (34.1%; by serology only) and bats (13.5%; by serology only) are likely more vulnerable to dengue virus infection than the other animals reported in

Table 2
Study characteristics and dengue positivity among bats.

Animal Types	Country	Location	Site Setting	Study Period	Dengue RT-PCR Positive Only (%); Serotype	Dengue Serology Positive Only (%); Serotype	Serological Test Type; Serotype Tested	Human Dengue Cases; Serotype	Dengue Serotype (Mosquitoes)	References
Bats	Mexico	Colima & Jialisco (Pacific Coast)	Urban/rural	2005		5/40 (12.5)	ELISA; Unspecified	2339 cases reported in 2005–2006		[46]
		Veracruz (Gulf Coast)		2006	4/30 (13.3); D2	4/46 (8.7)		7272 cases reported in 2005–2006; D2 outbreak in 2006		
Bats	Mexico	Calakmul Biosphere Reserve	Forest	2010–2011	4/85 (4.7); D2			Reported among human populations (20 km away); D1,2,4		[58]
Bats	Mexico	Montes Azules Biosphere Reserve	Urban/Rural	2011–2012	0/149	0/149	PRNT; Tested for D2/4 only	196 cases reported in 2011–2012; Endemic with D1,2		[52]
		Morelos, Central Mexico		2012	0/91	0/91		764 cases reported in 2012 in the city; Endemic with D1,2		
Bats	Mexico	Progreso, Hidalgo (Cave Roost)	Urban/rural	2014–2015	8/16 (50); D2 (NS5)*					[48]
Bats	China	Shankouxiang, Mei Wen Village, Hainan Island	Urban/rural	1995	23/56 (41.1%)	16/20 (80); D2	ELISA; Tested for D1/2/3/4	Outbreaks recorded from 1980 to 1982, 1986–1991 (Coastal region of dengue region), with each outbreak affecting hundreds of thousand	1/3 lots [1/30 (3.3) – 10/30 (33.3)] of female <i>Aedes aegypti</i> captured from endemic area positive by RT-PCR (NS1)	[47]
Bats	French Guiana	le Camp du Tigre	Forest	2001, 2006, 2007	19/125 (15.2); D1,2,3			Active outbreaks of D1/2/3 since 1991, sporadic detection of D4; Dengue endemic.		[44]
		Site of Sain Georges de l'pyapock	Edge of forest & rural area	2006, 2007	D1.G1 (C/prM)*	0/16		Dengue practically absent in this rural site.		
Bats	Costa Rica	La Virgen from Sarapiquí (rural households)	Urban/rural	2013–2014	6/102 (5.9); D2.Asian/American (C/prM)**; D3, D4.G2 (C/prM)*	23/75 (30.7); D1,2,3	PRNT; Tested for D1/2/3/4	16.7% seroprevalence (4/24)	D3 (<i>Culex</i> sp)	[51]
		Nicoya (semi-urban households)			6/98 (6.1); D2.Asian/America (C/prM), D4.G2 (C/prM)*	14/82 (17.1); D1,2,3,4		82.6% seroprevalence (19/23)		
		Central Valley (urban households)			16/118 (13.6); D1, D2.Asian/America (C/prM)**; D4.G2 (C/prM)*	14/84 (16.7); D1,2,3		8.3% seroprevalence (1/12)	D1 (<i>Ae aegypti</i>), D2.Asian/American (C/prM); <i>Culex</i> sp)*	
Bats	Mexico	Parque Hundido	Urban/rural	2010	0/59 (Flavivirus RT-PCR targeting NS5)	14/59 (23.7); D1,2,3,4	PRNT; Tested for D1/2/3/4	Presence of all 4 serotypes		[55]
		Parque Metropolitano			0/42	2/42 (4.8); D1,2,4				
		Acuaparque			0/21	2/21 (9.5); D1,2,4				
		Merida zoo			0/14	8/14 (57.1); D1,2,3,4				
		Santa Gertrudis Copo			0/4	0/4				

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Table 2 (continued)

Animal Types	Country	Location	Site Setting	Study Period	Dengue RT-PCR Positive Only (%); Serotype	Dengue Serology Positive Only (%); Serotype	Serological Test Type; Serotype Tested	Human Dengue Cases; Serotype	Dengue Serotype (Mosquitoes)	References
Bats	Trinidad	Unknown	Unknown	1972–1974		126/857 (14.7); D2	HI; Tested for D2 only			[61]
Bats	Grenada	All 6 parishes of Grenada	Urban/rural	2015		0/50	PRNT; Tested for D1/2/3/4	All 4 serotypes documented between 2000 and 2003; Dengue endemic.		[57]
Bats	Costa Rica	Puntarenas & Liberia	Urban/rural	1998		12/53 (22.6); D1,2	PRNT; Tested for D1/2/3/4	Active outbreaks of dengue, all 4 serotypes have been isolated in the past 6 years of the study.		[49]
Bats	Ecuador	Tena				3/10 (30); D2,3		Unknown		
Bats	Mexico	Cuiztzmala River Basin, Jalisco	Forest	2014	0/83 (Tested against flavivirus; NS5)			DENV reported in humans		[59]
Bats	Brazil	São José do Rio Preto, São Paulo State	Urban/rural	2014–2017	0/64 (Tested against flavivirus; NS5)	0/46	HI; Tested for D1/2/3/4	High incidence of arbovirus infection in humans		[50]
Bats		Barreiras, Bahia State			0/39 (Tested against flavivirus; NS5)	0/27		Known occurrences of DENV		
Bats	Columbia	San Carlos & Ayapel, Córdoba & Sucre	Urban/rural	Before 2019	2/286 (0.7); D2 (NS5)*					[45]
Bats	Singapore	Unknown	Urban/rural	Before 2019		14/106 (13.2); D2 (NS1)	Luciferase LIPS; Tested for D2 NS1 antigen only			[54]
Bats	Australia	Townsville, Northern Queensland	Urban/rural	1954		4/17 (23.5); D1	Mouse protection test; Tested for D1 only	Extensive epidemic from Dec 1953 – Mar 1954, sporadic cases from Dec 1954 – Nov 1955.		[53]
Bats	Australia	Townsville, Northern Queensland	Coastal	1954		4/28 (14.3); D1,2	Mouse protection test; Tested for D1/2 only	Dengue outbreak in the early months of 1954		[60]
Bats	India	Bankura District, West Bengal	Urban/Rural	1973		1/91 (1.1); D3	HI; Tested against D1/2/3 only			[56]

* Phylogenetic analysis linked to human cases.

** Phylogenetic analysis linked to both human cases and mosquitoes.

the literature. Dengue virus has been known to infect NHPs such as monkey, which serves as an efficient amplification host for enzootic dengue transmission [13,14,16]. However, based on this review and existing studies [14], not all species of primates may be susceptible to dengue virus infection. As the only natural vertebrate host of dengue apart from humans, their susceptibility to dengue virus has been extensively leveraged as models for studies on dengue pathogenesis and therapeutic interventions [81,82]. Rhesus macaque (*Macaca mulata*), cynomolgus macaque (*Macaca fascicularis*), green monkeys (*Cercopithecus aethiops*) and yellow baboons (*Papio cynocephalus*) to a less extent, are among species commonly used in experimental studies [13,82]. This study corroborates the infectability of these monkey species through serological identification of dengue exposure. Interestingly, these same species – baboons (*Papio*) and Macaque (*Macaca*) – were found to be more behaviorally and ecologically resilient, enabling their survival even in urban areas in the face of anthropogenic stressors [83]. African green monkeys also reportedly sustained longer viremia when infected with human-endemic strains of DENV-2 as compared to sylvatic DENV-2

[84]. This represents a longer viremic duration that could allow infection of more vectors in the event the monkeys are infected, serving as an amplification host. However, findings cannot be generalized to species of monkeys that were not tested.

While the long-tailed macaque (*Macaca fascicularis*) and banded surili (*Presbytis femoralis*) are both native to Singapore [85,86], only the former – known to be more susceptible to dengue and adaptable to human environments – is commonly seen in Singapore, numbering some 1500 individuals in the population. Globally, Brazil, Madagascar, Indonesia and the Democratic Republic of Congo are home to two-thirds of 504 primate species in existence. Yet, all four countries are carrying out massive destructions to their primate's habitats with human activities [87], which could force NHPs and humans to interface at increasing levels.

Kato et al. demonstrated a spillback event in cynomolgus macaques in the Philippines, proving that NHPs can be involved in the human/urban DENV cycle [40]. However, their potential to act as viable reservoirs depends on whether they can sustain sufficient viremia to enable

Dengue in Animals

11.1%

DENGUE POSITIVITY IN ALL ANIMALS TESTED

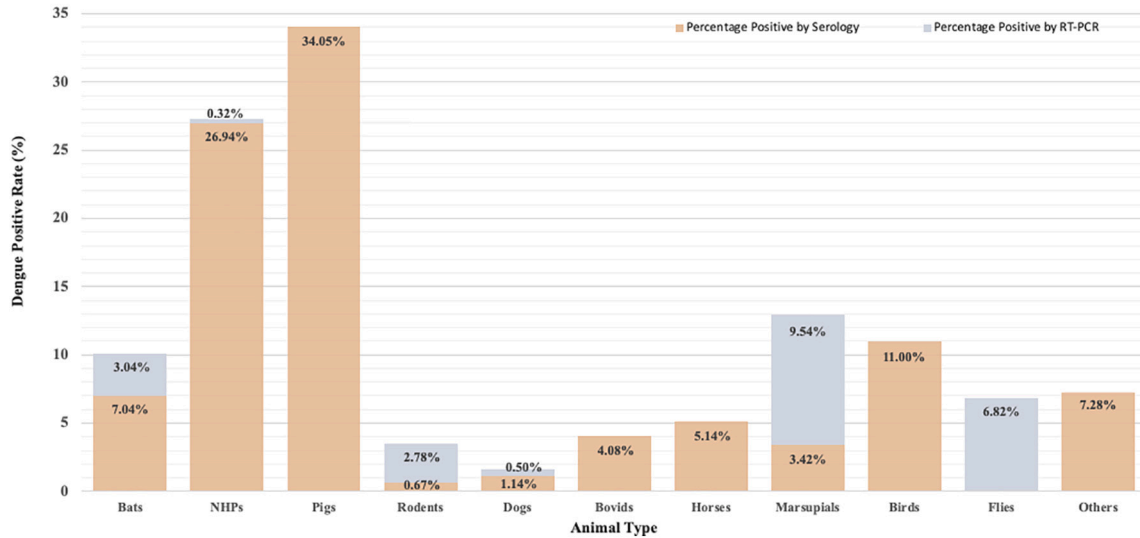


Fig. 3. Dengue positivity rate of different animal types detected by PCR and serology-based assays. NHP: Non-human primates; Others: Shrew, Sloths, Acouchy, Agouti, Porcupine, Armadillo and Kangaroo.

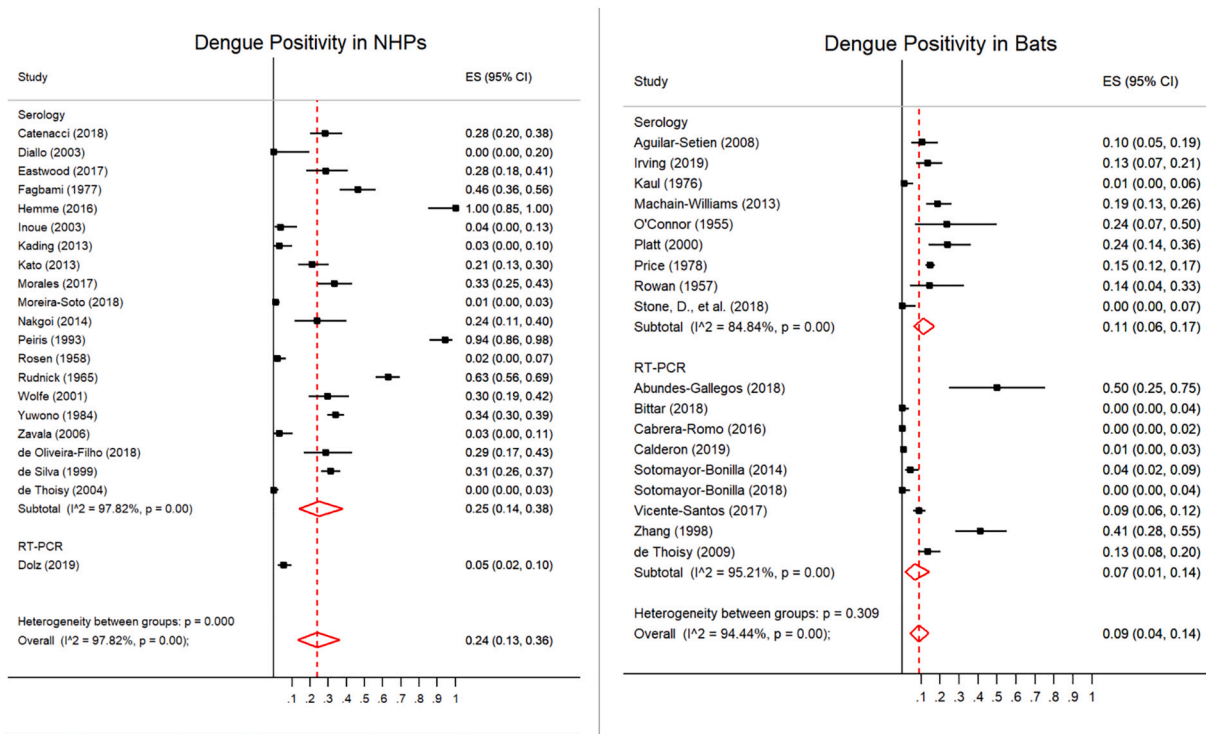


Fig. 4. Forest plot of dengue prevalence in NHPs. Forest plot of dengue prevalence in bats.

mosquitoes to become infected, causing subsequent transmission to NHPs. Further investigation of their potential for zoonotic transmission of urban strains should be conducted on NHPs living near human settlements and vector studies. The spillover of sylvatic DENV strains, albeit uncommon, have been documented on several occasions in individuals who entered the forests. With persistent encroachment of human settlements into undeveloped forests, the increasing occurrence

of spillover from the sylvatic cycle can endanger local populations active near the zones of emergence as well.

Bats are evolutionary successful creatures that are widely distributed globally. At least one species in each of the 19 families constituting the order Chiroptera is known to roost in buildings [88]. *Myotis yumanensis*, *M. lucifugus*, *Tadarida brasiliensis* [89,90] (one of the most abundant species in North America), *Eptesicus fuscus* [91] and *Pteropus lylei* [92]

Table 3
Study characteristics and dengue positivity among other animals.

Animal Types	Country	Location	Site Setting	Study Period	Dengue RT-PCR Positive Only (%); Serotype	Dengue Serology Positive Only (%); Serotype	Serological Test Type; Serotype Tested	Human Dengue Cases; Serotype	Dengue Serotype (Mosquitoes)	References
Bat Flies	Mexico	Progreso, Hidalgo (Cave Roost)	Urban/rural	2014–2015	38/557 (6.8%) to 342/557 (61.4%); D2 (NS5)					[48]
Rodents	French Guiana	le Camp du Tigre	Forest	2001, 2006, 2007	28/109 (25.7); D1,2,3,4			Active outbreaks of D1/2/3 since 1991, sporadic detection of D4; Dengue endemic.		[44]
Marsupials	French Guiana	le Camp du Tigre	Forest	2001, 2006, 2007	5/21 (23.8); D3,4 D3.G5 (C/prM)*, D4.G1 (C/prM)* 39/273 (14.3); D1,2,3,4 D2.American-Asian (C/prM)*, D2. Native American (C/prM)*, D3.G5 (C/prM)*, D4.G1 (C/prM)*			Active outbreaks of D1/2/3 since 1991, sporadic detection of D4; Dengue endemic.		
Birds (seashore)	Australia	Site of Sain Georges de l'pyapock Townsville, Northern Queensland	Edge of forest & rural area Coastal	2006, 2007 1954	0/15	8/38 (21.1); D1	Mouse protection test; Tested for D1 only	Dengue practically absent in this rural site. Dengue outbreak in the early months of 1954		[60]
Birds	India	West Bengal, Bankura Distict	Urban/ Rural	1973		3/104 (2.9); D1	HI; Tested for D1/2/3 only			[56]
Rodent Shrew Sloths	Brazil	Atlantic Forest, Bahia, Northeast Brazil, municipalities of Ilheus and Una	Forest & Urban/rural (enclosure)	2006–2014		14/29 (48.3); D1,2,3,4	HI; Tested for D1/2/3/4	D1,2,3,4 are endemic to both municipalities with sporadic outbreaks		[36]
Pigs	Malaysia	Penang	Urban	1962–1964		7/34 (20.6); D2	PRNT; Tested for D2 only			[35]
House Shrew						1/18 (5.6); D2				
Acouchy Agouti	French Guiana	Petit Saut Hydroelectric Dam	Forest	1994–1995		0/29 1/29 (3.4); D2	PRNT; Tested for D2 only	Dramatic increase in cases over the past 30 years, epidemic in 1991–1992 with up to 3000 cases.		[37]
Porcupine						2/42 (4.8); D2				
Paca						0/17				
Rat						0/39				
Opposum						1/99 (1); D2				
Sloth						0/55				
Armadillo						3/60 (5); D2				
Anteater						0/26				
Kinkajou						0/9				
Coati						0/4				
Tayra						0/3				
Collared Peccary						0/3				
Brocket Deer						1/10 (10); D2				

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Table 3 (continued)

Animal Types	Country	Location	Site Setting	Study Period	Dengue RT-PCR Positive Only (%); Serotype	Dengue Serology Positive Only (%); Serotype	Serological Test Type; Serotype Tested	Human Dengue Cases; Serotype	Dengue Serotype (Mosquitoes)	References
Buffalo+	Democratic Republic of Congo & Gabon	Garamaba National Park & Lope National Park	Forest	1994–2002		1/24 (4.2); D2	PRNT; Tested for D2 only			[30]
Duiker+	Democratic Republic of Congo	Ituri Rain Forest	Forest	1991–1992		0/33				
Elephant	Zambia, Democratic Republic of Congo, Chad & Central African Republic	Lungunya, Garamba National Park, Odzala National Park, Zakouma National Park, Gobounga Bai, Dzanga-Sangha National Park	Forest	1991–2002		0/31				
Pigs	India	Darbhanga Patna Begusarai Nawada Dhanbad Singhbhum Hazaribagh Ranchi	Urban/rural	1978–1979		5/16 (31.3); D2 26/40 (65); D2 8/16 (50); D2 13/35 (37.1); D2 11/32 (34.4); D2 3/14 (21.4); D2 50/111 (45); D2 64/140 (45.7); D2	HI; Tested for D2 only			[69]
Dog	India	Uttar Pradesh, Bareilly	Urban/rural	Before 1995		16/104 (15.4); D2 20/170 (11.8); D2 27/170 (15.9); D2 26/333 (7.8); D2	HI; Tested for D2 only			[72]
Pig						10/252 (4); D2				
Horse						0/252 0/168				
Buffalo+						0/708				
Goat+										
Cattle+										
Sheep+										
Rodents	Mexico	Cuitzmala River Basin, Jalisco	Forest	2014	0/713 (Tested against flavivirus; NS5)		ELISA; Tested for D2 only	DENV reported in humans		[59]
Rodents	Mexico	Merida, Residential Housings	Urban/ rural	2011–2012		5/161 (3.1); D2	PRNT; Tested for D2/4 only	Dengue endemic with outbreaks		[64]
Dogs (Domestic)	Thailand	Nakhon Sawan Province (Urban City) Rayong Province (Rubber Plantation) Koh Chang (Tourist Island)	Urban/rural	2008–2009 2009–2010 2012	6/1057 (0.6); D2,3 (C/prM)* 1/174 (0.6); D3 (C/prM)* 0/71			Dengue endemic		[63]
Equines	Brazil	Nhecolândia Sub-region of Pantanal	Urban/rural	2009–2010		8/760 (1.1); Screened against	Screened with ELISA, 396 positive ELISA samples tested using PRNT;	Dengue endemic		[71]

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Table 3 (continued)

Animal Types	Country	Location	Site Setting	Study Period	Dengue RT-PCR Positive Only (%); Serotype	Dengue Serology Positive Only (%); Serotype	Serological Test Type; Serotype Tested	Human Dengue Cases; Serotype	Dengue Serotype (Mosquitoes)	References
Sheep+						flavivirus; D1,2,4 0/238; Tested against flavivirus Caimans	Tested for D1/2/3/4 for PRNT ELISA 0/61; Tested against flavivirus		ELISA	
Cattle+	Sicily, Italy	Madonie, Mazara del Vallo, Casteltermini, Castelvetro, Acamo, Canicattì & Palermo	Rural	1969		1/410 (0.2); D1	HI; Tested for D1 only			[74]
Sheep+						1/130 (0.8); D1				
Goat+	Australia	Junee	Unknown	1954		0/27	PRNT; Tested for D1/2 only	7/14 (50) Seropositivity in Neutralization Test. 110/142 (77.5) and 103/141 (73) seropositivity by to D1 & D2 respectively		[76]
Horses		Rockhampton		1955		4/14 (28.6); D1		Individuals had been screened for Group B virus using MVE HI.		
		Mt Surprise		1955		2/2 (100); D1				
		Gympie		1960		1/1 (100); D1/2				
Cattle+		Innisfail		1954		2/5 (0.4); D1				
Kangaroo		Tambo		1958		0/1				
		Western Australia		1958		5/12 (41.7); D1				
		Muckadilla				1/1 (100); D1				
		Tambo				1/1 (100); D1				
		South-east Queensland		1960		6/8 (75); D1/2				
Cockatoo	South Pacific Islands	Unknown	Urban/Rural	1959		1/2 (50); D1		Outbreaks in 2008–2009, 2012–2013 (>10, 000 cases); D1. Circulation of all 4 serotypes in 2013. D1 epidemic in 2006–2007 (circulation in 2013–2017). D3 circulation in 2013		[68]
Horses		New Caledonia (Includes imported horses)		2015		10/163 (6.1); D1	Screened with ELISA, Positive samples tested with MNT; Tested for D1 only in MNT			
		Marquesas Islands, French Polynesia (Includes imported horses)		2016		10/130 (7.7); D1				
Domestic Animals (Mammals & Birds)	Egypt	Cairo Slaughter Abattoir	Urban/Rural	1969		74/964 (7.7); D1	HI; Tested for D1 only	159/1133 (14%); 4/1133 (0.3) (accounted for WNV) seropositivity against D1 in 1969 survey. Dengue epidemics in 1927, 1928 and 1937		[73]
Ducks	India	Ranchi viz. Kanke, Bihar	Urban/Rural	1981		34/64 (53.1); D2	HI; Tested for D2 only			[70]
Fowls		Ranchi viz. Kanke, Bihar				30/81 (37); D2				
Sparrows		Poultry Farm, Kanke, Bihar				1/16 (6.25) – 16/16 (100); D2				
Fowls		Hotwar, Bihar				26/63 (41.3); D2				
Fowls		Daily Market, Bihar								

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Table 3 (continued)

Animal Types	Country	Location	Site Setting	Study Period	Dengue RT-PCR Positive Only (%); Serotype	Dengue Serology Positive Only (%); Serotype	Serological Test Type; Serotype Tested	Human Dengue Cases; Serotype	Dengue Serotype (Mosquitoes)	References
Geese	Czechoslovakia	South Moravia	Urban/rural	1972		33/68 (48.5); D2	HI; Unspecified			[65]
Ducks						0/196				
Hens						0/141				
Birds	Czechoslovakia	South Moravia	Urban/rural	1973		0/280	HI; Unspecified			[66]
Birds	India	Shimoga district, Karnataka	Urban/rural	1959–1962		93/759 (12.1); D1,2,3,4	HI (17 sera positive for HI tested for PRNT); Tested for D1/2/3/4			[67]
Birds	Uganda	Bunyoro, Karmoja, Acholi, Ankole & West Mengo	Forest (Including savannah)	1970		15/221 (6.8); Unknown	HI; Unspecified			[75]
Birds	Brazil	Mangal das Garças Natural Park in Belém, state of Pará	Urban	2015		0/85	HI; Tested for D1/2/3/4			[62]

+ Bovids.

* Phylogenetic analysis linked to human cases.

are just some examples of bats staying in close proximity with human population in both rural and urban settings [88]. Of more than a thousand species of bats in existence, there are at least 25 native species that roost in both natural and man-made habitats in Singapore [85,93]. They include Old world fruit bats (*Pteropodidae*), horseshoe bats (*Rhinolophidae*), false vampires (*Megadermatidae*), hollow-faced bats (*Nycteridae*), free-tailed bats (*Molossidae*) and evening bats (*Vespertilionidae*). Specifically, the lesser dog-faced fruit bat (*Cynopterus brachyotis*) and whiskered myotis (*Myotis muricola*) are reported to commonly roost in residential estates [94]. In a study involving *Cynopterus brachyotis* captured in Singapore, dengue exposure by D2 anti-NS1 antibodies was noted in 13.2% of bats [54].

Vicente-Santos et al. made an interesting conjecture on the infection of bats via oral ingestion of mosquitoes when they could not find evidence supporting bat infection through infected mosquito bite [51]. While evidence is still very limited [51], this could explain the conflicting observation of DENV presence in bats, which has been proven on several occasions [51,58], and the general lack of substantial findings from experimental inoculation studies [95–97], which cast doubts on their role as competent hosts/reservoirs. Apart from non-human primates, bats are the most extensively studied for presence of dengue infection, with the most studies conducted involving RT-PCR. Although the proportion of bats positive by RT-PCR was higher than that in non-human primates, it could be attributed to the greater number of bat studies than employed RT-PCR as compared to NHP studies. This study noted a lower proportion of bats positive for dengue by RNA detection as compared to serology based on both the inclusion of studies utilising more than one diagnostic platform (serology vs RT-PCR: 13.5% vs 5.8%), and the meta-analysis of studies based on one platform of detection (serology vs RT-PCR: 11% vs 7%). This indicate prior dengue infections in bats sampled instead of active ones. Of the six studies that conducted both serological testing and RT-PCR, three studies [46,47,51] yielded comparative results while the remaining three [50,52,55] did not detect any dengue positivity for comparison. Overall, the infection and amplification mechanism of DENV in bats warrants deeper investigation. The detection of DENV in bat flies by RT-PCR in a single study also highlights the importance of studying hematophagous bats and the interactions between bats and ectoparasitic organisms in infection. In view of the diverse range of bat species in existence compared to the few tested, species staying near/in the urban and rural areas may still play a role as reservoirs conserving dengue transmission.

While the study notes a high seroprevalence of DENV in pigs, the available literature were dated some time back and utilized HI predominately. Domestic pigs common in rural setting may act as one of the potential reservoir for dengue epizootic transmission resulting in increased spillover and epidemic in rural human population, similar to Japanese encephalitis virus (JEV) [98]. Pigs are also susceptible to Zika – a flavivirus similar to dengue – infection and can generate viremia upon experimental inoculation [99]. A relatively high DENV seroprevalence was also observed in marsupials, well-known to be primary reservoirs of Ross River virus, a mosquito-borne alphavirus [100]. No in-depth studies has been conducted to explore the potential of pigs and marsupials as DENV reservoirs hitherto. Dengue positivity was also observed in birds, dogs and rodents, which are animals commonly found in the urban setting. The high seroprevalence observed in birds ought to also be interpreted with caution given that detection was mainly done using HI and birds are known to be reservoirs of JEV [101]. Given the hypothesized feeding plasticity of *Aedes albopictus*, plausible DENV presence in the blood of these animals means that mosquitoes can be infected when biting them [102]. By extension, abundance of these animals in urban environments translates to heightened risk of dengue exposure in humans who become infected through mosquito bites as well. Furthermore, studies have also observed a wide feeding host range of the mosquitoes vectors that transmit dengue efficiently, namely *Aedes albopictus* [103] and *Aedes aegypti* [104].

5. Limitations

This systematic review is limited by a lack of homogeneity across assays employed in the respective studies. Inconsistency in assays used, differential laboratory setting and sample handling may have affected the results consolidated in our study as seen with the high heterogeneity in dengue prevalence results from our meta-analysis. In addition, usage of different RT-PCR and serological assays from different time periods, different testing strategies whereby some studies only tested specific DENV serotypes, or tested for viral genera were also contributing factors.

The capture of sufficient sample size and a viable specimen volume for each animal are implicated by time, environmental constraints, and size of the animal caught. This is not an infrequent observation in the available literature, as seen from one selected study [70] having to pool serum from 16 sparrows to constitute one bird sample. Insufficient sample volumes collected or a small overall sample size in certain studies hence, limit their analysis given that a larger sample size increases the significance level of the findings and produce more generalizable results. This subsequently affects the accuracy of our review in generalizing the trends across specific animal types.

Third, many of the reports lacked evidence of live DENV, which is best confirmed by virus isolation, the gold standard in dengue diagnosis. Only two studies [48,63] in the available literature attempted and successfully isolated virus from its RT-PCR positive samples. While positivity by serology or RT-PCR test constitute indirect evidence of dengue presence in animals, they do not confirm an active infection and/or circulation of the virus among animal population. The dearth of studies that have successfully isolated virus from the animal samples, or even attempted to do so demonstrates a lack in substantial evidence supporting the role of animals as potential reservoirs in the urban dengue transmission cycle.

Furthermore, there have been limited studies exploring DENV infection and replication in bats and animals that are commonly found in the urban setting. Without strong evidence of sustained dengue virus replication in animals, it is uncertain if animals currently can or as they evolve over time may play a role as potential reservoirs or are simply incidental hosts in the dengue transmission cycle. While the inability to generate sufficient viremia, hence, adequate antibody titers could underlie any lack of genetic material or antibody detected, experimental inoculation/immunological studies are still required to investigate the mechanism of DENV infection and the viability of replication in different animal types to consider their true plausibility as reservoirs.

Lastly, cross-reaction with other flaviviruses should not be ruled out in the serological assays. Individuals infected with a flavivirus are well known to produce broadly reactive antibodies that are highly cross-reactive with other JEV serocomplex viruses [40,71]. The ELISA and HI assays do not differentiate between broadly reactive or serotype-specific antibodies, nor differentiate neutralizing antibodies like PRNT. Yet, most of the studies compiled by this systematic review had ascertained dengue positivity through serological tests and did not follow-up with diagnostic tests to exclude other viruses of the same genera. Taken together, the dengue seropositivity observed by our study may represent an inflation of the actual dengue presence in animals. Results should be interpreted with caution.

6. Conclusion

Dengue virus is capable of infecting a number of animal species, however, their role as amplifying reservoirs is uncertain due to various limitations in the evidence. It seems plausible that some animals in an urban settings may play an indirect role in dengue ecology, possibly even with low titers of viral load. However, this requires more investigation.

Author contributions

GSXW carried out the search, extraction of data and data analysis. PJ checked and analyzed the data and wrote the first manuscript draft. Both GSXW and PJ edited subsequent versions of the draft. GCG conceived of the study. SJAL and GCG reviewed drafts of the manuscript and suggested changes.

Data availability

All data generated or analysed during this study are included in this published article (and its Supplementary Information files).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.onehlt.2021.100216>.

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