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Serological and molecular detection of dengue virus in animals: A systematic review and meta-analysis

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SUMMARY

Introduction: Dengue is a vector-borne disease, especially important in tropical and subtropical areas. The first presentation of many arboviral diseases occurred mainly in animals, including multiple *Alphaviruses* and *Flaviviruses*, such as dengue.
Objective: To determine the serological and molecular frequency of the dengue virus in animals.
Methods: A systematic literature review was carried out in five databases for the proportion of animals infected with dengue, defined by molecular and serological tests. A meta-analysis was performed using a random-effects model to calculate the pooled prevalence and 95% confidence intervals (CI). Cochran?s Q test and the I2 statistic were used to assess the heterogeneity between the two studies.
Results: The presence of dengue in bats, primates, birds, sheep, horses, cattle, pigs, rodents and buffaloes, according to serological methods, had a prevalence of 10%, 29%, 8%, 1%, 11%, 0%, 49%, 2%, 7%, respectively. According to molecular methods, the presence of dengue in bats had a seroprevalence of 6.0%.
Conclusion: The present study confirms the presence of the Dengue virus in a large group of animal species, with potential implications as possible reservoirs of this virus, raising the possibility of zoonotic transmission.

Keywords: Animals, dengue, virus, zoonosis, systematic review, and meta-analysis.

INTRODUCTION

Dengue continues to be a significant public health threat and the most important arboviral disease in terms of morbidity and mortality globally [1, 2]. The Dengue virus (DENV) corre-

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sponds to the Dengue serocomplex, *Flavivirus* genus, Flaviviridae family. There are four distinct but closely related serotypes: DENV1, DENV2, DENV3, and DENV4; these periodically cross endemic and hyperendemic areas, and all cause the disease known as Dengue [3].

Each of the four Dengue serotypes generates a unique immune response to infection in the host. They are distributed throughout tropical and subtropical regions worldwide. The four serotypes are genetically similar, share approximately 65% of their genome, and are transmitted to non-human primates (jungle form) and humans (human form), mainly by the *Aedes* genus mosquito [4].

The main vectors of the DENV are the *Aedes aegypti*, *Aedes albopictus,* and *Aedes vittatus* mosquitoes, which have been recently reported in the Americas [5]. They live in urban habitats where they reproduce in containers with accumulated water, and their feeding is during the day; the periods of the bites are intensified in the early morning and the evening before it gets dark. The cycle comprises four stages: egg, larva, pupa, and adult, lasting 8 to 10 days [6].

Transmission of the virus occurs mainly through the bite of infected female mosquitoes. After biting the host, the mosquito expels saliva filled with the virus into the human?s blood. The incubation period for the virus lasts between 4 and 10 days, and an infected mosquito can transmit the pathogen for a lifetime. Asymptomatic and symptomatic infected humans are the primary carriers of the virus, and mosquitoes become infected by biting them. After the appearance of the first symptoms, these infected people can transmit the infection for 4 or 5 days, 12 days maximum, to *Aedes* mosquitoes [7]. In addition to the urban cycle, there is at least one jungle cycle in which other vectors can potentially participate, where animals can play an important role [8].

Some studies have identified DENV in animals; this is the case of a study in which 293 equine serum samples were analysed in the French Pacific Islands, where they found 7.7% positivity for Dengue virus serotype 1 (DENV1) [9]. In Thailand, in a study of 38 macaques (*Macaca nemestrina*), it was determined by serology that 24% were positive for DENV [10]. In another study carried out in Costa Rica and Ecuador, neutralizing antibodies to serotypes 1 and 2 (22.6%) and 3 (30.0%) of the Dengue virus were detected in bats [11]. Finally, in another study in Colombia, in the departments of Córdoba and Sucre, they sampled 286 non-hematophagous bats, *Carollia perspicillata* and *Phyllostomus decoloran*, where they found that the amplicons showed a high similarity with Dengue virus serotype-2 (DENV-2), being the first evidence of the DENV-2 genome in bats from the Colombian Caribbean [12].

Concerning the clinical findings in animals infected with dengue, there are no appropriate animal models for the study of the physio-pathogenesis and the clinical manifestations of the disease, nor there are studies as references for the evaluation of specific pharmacological treatment. No animal suffers clinical manifestations similar to those expressed by humans, whether infected by mosquitoes or experimentally [8].

DENV is a severe disease with epidemiological, social, and economic impact, which has become a growing problem for global public health. In addition, it is an emerging and re-emerging disease of greater magnitude and importance due to its tremendous economic impact on the exposed population. Therefore, it represents a severe public health problem in the American Region [13].

The disease in humans can be asymptomatic or present symptoms that vary from mild fever to impatient fever, accompanied by severe headache, pain behind the eyes, pain in muscles and joints, and erythema; it can also progress to severe forms, mainly characterized by shock, respiratory distress and severe organ damage [14].

In most tropical Americas, Dengue epidemics occur periodically in different countries, especially Brazil, Colombia, and Venezuela [15]. The four virus serotypes are present in America, and their cocirculation was reported in Brazil, Guatemala, and Mexico. This simultaneous circulation of two or more serotypes increases the occurrence of severe disease cases. Due to its population size, Brazil has the highest number of registered cases, followed by Mexico, Nicaragua, and Colombia, with 106,066 cases reported in 2019 [10]. Diagnostic methods for the detection of DENV in humans include RT-PCR (Reverse transcriptase-polymerase chain reaction), ELISA (Enzyme-Linked Immunoassay), nonstructural protein one antigen (NS1 antigen), hemagglutination inhibition (HI), and immunochromatography [15].

Demographic, social, and environmental factors, such as unplanned urbanization, migration, cultural aspects, housing conditions, and the quality of

health service provision, have influenced the spread of the vector, increasing the incidence and appearance of the disease in new geographical areas.
Climate change influences the DENV, as an increase of 1 to 2 degrees of temperature extends the development of the vectors, which are poikilothermic. This study aims to determine the serological and molecular frequency of the Dengue virus in animals, according to species, countries, and serotypes, and to analyse the average viral load of the virus in animal reservoirs.

METHODS

Registration and reporting
A summarised version of the protocol for this systematic review was uploaded to the International Prospective Register of Systematic Reviews (PROSPERO), and we drafted our results using the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement [16].

Search strategy and databases
The search strategy followed the Peer Review of Electronic Search Strategies (PRESS) Checklist [17]. The search terms were based on MeSH and accessible terms for "Dengue animals", "Dengue reservoirs", "Prevalence Dengue animals", and "Prevalence Dengue reservoirs". Afterwards, the search formula was adapted for all databases without language restrictions. We run the systematic search (March 10, 2023) through the following databases: PubMed, Scopus, Web of Sciences, LILACS and Scielo. We detailed the complete search strategy in Table 1.

Table 1 - Search strategies.

Source	*PubMed*
Search	*Formula*
#1	Arbovirus [MH] OR Dengue [MH] OR "DENV*" [TIAB] OR ("mammals" [TIAB] AND "animal*" [TIAB]) OR "monkeys*" [TIAB]
Source	*Scopus*
Search	*Formula*
#1	TITLE-ABS-KEY ("DENV*" OR ("mammals" W/3 "monkeys*") OR "animals*")
Source	*Web of Science*
Search	*Formula*
#1	TI=("DENV*" OR ("mammals" NEAR/3 "animals") OR "mammals") OR AB=("DENV*" OR ("mammals" NEAR/3 "animals") OR "mammals") OR AK=("DENV*" OR ("mammals" NEAR/3 "animals") OR "mammals") OR KP=("DENV*" OR ("mammals" NEAR/3 "animals") OR "mammals") OR TS=("DENV*" OR ("mammals" NEAR/3 "animals") OR "mammals")
Source	*Scielo*
Search	*Formula*
#1	(Dengue) AND (Animals)
Source	*LILACS*
Search	*Formula*
#1	(DENV* OR (mammals adj3 animals) OR mammals).ti. OR (DENV* OR (mammals adj3 animals) OR mammals). ab. OR (DENV* OR (mammals adj3 animals) OR mammals).kw.

Study selection and data extraction
We performed each phase of the study selection process independently and by at least two authors. The eligibility criteria were:
1) observational studies reporting the
2) infection by DENV in animals with serological or molecular tests.

ELISA, microneutralisation test (MNT), plaque reduction neutralisation test (PRNT), HI, complement fixation (CF), and NS1 antigen were considered for serological tests, and RT-PCR for tests based on molecular biology. We excluded narrative reviews, scoping reviews, systematic reviews and conference abstracts. References with incomplete information were also excluded. We removed duplicates with Rayyan QCRI © [18]. Two authors screened the remaining references by titles and abstracts in Rayyan. The authors independently assessed the full text of the relevant references to be included. We resolved any conflict or discrepancy in any phase of the study selection process by consensus. Two authors independently extracted data using a standardized data extraction sheet built-in Google Sheets ©. The following information was extracted: author, publication type, publication date, publishing institution, country, sample size, infected animals, method of detection, and serological or molecular tests.

Risk of bias and publication bias
The quality assessment process was performed independently by two authors. We used the adapted

version of the Newcastle-Ottawa Scale for cross-sectional studies (NOS-CS). A score ≥7 stars was considered a low risk of bias; otherwise, the study was considered to have a high risk of bias [19]. The publication bias assessment was not performed because it is not recommended for proportional meta-analysis. After all,

1) conventional funnel plots and Egger?s test are inaccurate for these analyses, and
2) there is no evidence that proportions adjust correctly to funnel plots or Egger?s tests [20, 21].

Data analysis

STATA 17.0 © was used for performing statistical analysis. We conducted a pooled analysis of the prevalence of DENV-infected animals according to serological or molecular tests. The 95% Confidence Intervals (CI) for the proportions reported in each study were calculated using the Clopper-Pearson Method. The Freeman-Tukey Double Arcsine Transformation was used as the variance stabiliser. We used a random-effects model (Dersimonian and Laird) for the quantitative analysis. We assessed the between-study heterogeneity using Cochran?s Q test and the I^2 statistic. Values equal to or greater than 60% were defined as high heterogeneity for the I^2 statistic, and p-values <0.05 are considered indicators of heterogeneity in Cochran?s Q test. In addition, we carried out subgroup analysis using the serological method and continents. Sensitivity analyses were performed using only studies with a low risk of bias.

RESULTS

Selection and characteristics of the studies

The systematic search retrieved 3921 records; after removing duplicates, 2352 records remained. After excluding articles by title and abstract and assessing their full-text documents, 54 studies were identified as eligible for the qualitative and quantitative syntheses [9-12, 22-71]. The flow diagram summarizing the study retrieval is shown in Figure 1.

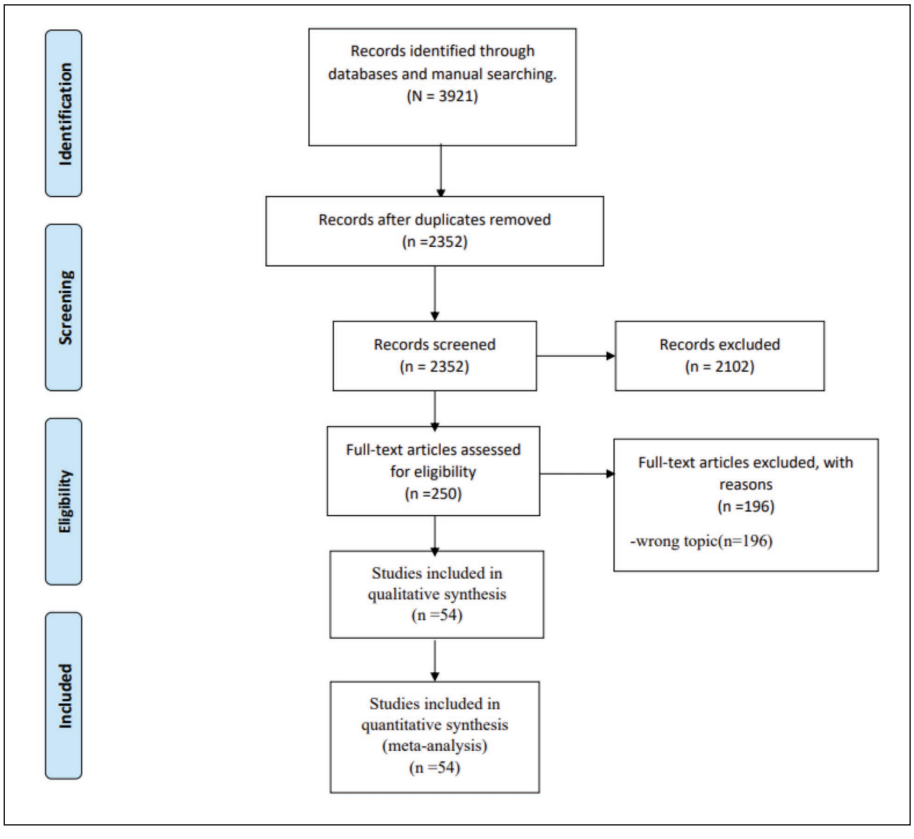

Figure 1
PRISMA Flow Diagram.

The characteristics of the studies are presented in Table 2. The included studies were conducted between 1958 and 2020 with 11824 animals. Regarding evaluating the quality of the studies with the NOS, 48 were at a low risk of bias, and the remaining six were at a high risk of bias (Table 3).

Bats

Serological methods

The presence of dengue in bats according to serological methods was evaluated in 13 studies (n=2688), with a seroprevalence of 10.0% (95% CI: 4.0%-17.0%; I^2=96.62%) (Figure 2). When subgroup analysis was performed according to the type of serological methods (Figure 3), it was found that bats evaluated by PRNT, ELISA and HI had a prevalence of 6.0%, 23.0%, and 3.0%, respectively. In the subgroup analysis according to continents, it was found that the bats evaluated in America and Asia had a prevalence of 8.0% and 24.0%, respectively (Figure 4). In their sensitivity analysis, no decrease in their heterogeneity was found, with a preva-

Table 2 - Characteristics of the included studies.

Author	*Year-Publication*	*Country*	*Type of animal*	*Serological method*	*Dengue serotype*	*N*	*n(+)*	*Molecular method*	*Dengue serotype*	*N*	*n(+)*
Zavala R et al.	2006	Venezuela	Non-human primates	HI	DEN-2	60	2	NR	NR	NR	NR
De Thoisy et al.	2004	French Guiana	Non-human primates	HI	DEN-2	145	25	NR	NR	NR	NR
			Opossum			99	1				
			Rodents			156	3				
			Sloths			55	0				
			Kinkajou			9	0				
			Coati			4	0				
			Tayra			3	1				
			Peccary			3	1				
			Deers			10	0				
			Armadillo			60	0				
			Anteater			26	2				
Aguilar-Setién A et al.	2008	Mexico	Bats	ELISA	DEN-1, DEN-2, DEN-3 and DEN-4	76	9	RT-PCR	DEN-2	30	2
Nakgoi K et al.	2014	Thailand	Non-human primates	PRNT	DEN-1, DEN-2, DEN-3 and DEN-4	38	9	NR	NR	NR	NR
Eastwood G et al.	2017	Kenya	Non-human primates	ELISA	DEN-1, DEN-2, DEN-3 and DEN-4	34	14	NR	NR	NR	NR
Calderon A et al.	2019	Colombia	Bats	NR	NR	NR	NR	RT-PCR	DEN-2	286	2
Kading R et al.	2013	Democratic Republic of the Congo, Gabon, Zambia, Chad, and the Central African Republic	Buffaloes	PRNT	DEN-2	24	1	NR	NR	NR	NR
			Duikers			40	0				
			Non-human primates			69	2				
			Elephants			34	0				

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Author	*Year-Publication*	*Country*	*Type of animal*	*Serological method*	*Dengue serotype*	*N*	*n(+)*	*Molecular method*	*Dengue serotype*	*N*	*n(+)*
Platt K et al.	2000	Costa Rica and Ecuador	Bats	PRNT	DEN-1, DEN-2, DEN-3 and DEN-4	63	15	NR	NR	NR	NR
Machain-Williams C et al.	2013	Mexico	Bats	PRNT	DEN-1, DEN-2, DEN-3 and DEN-4	140	26	RT-PCR	DEN-1, DEN-2, DEN-3 , DEN-4	140	0
Kilbourn A et al.	2003	Malaysia	Non-human primates	ELISA	NR	71	21	NR	NR	NR	NR
Vicente-Santos A et al.	2017	Costa Rica	Bats	PRNT	DEN-1, DEN-2, DEN-3 and DEN-4	318	51	RT-PCR	DEN-1, DEN-2, DEN-3 , DEN-4	318	28
Kading R et al.	2018	Uganda	Bats	PRNT	DEN-2	626	3	NR	NR	NR	NR
De Silva A et al.	1999	Sri Lanka	Non-human primates	ELISA	DEN-1, DEN-2, DEN-3 and DEN-4	304	95	NR	NR	NR	NR
Cigarroa-Toledo N et al.	2016	Mexico	Rodents	PRNT	DEN-2 and DEN-4	161	5	NR	NR	NR	NR
Beck C et al.	2019	France	Horses	ELISA	DEN-1	293	67	NR	NR	NR	NR
De Oliveira-Filho E et al.	2018	Brazil	Non-human primates	PRNT	DEN-1, DEN-2, DEN-3 and DEN-4	49	16	NR	NR	NR	NR
Sotomayor-Bonilla J et al. (A)	2014	Mexico	Bats	NR	NR	NR	NR	RT-PCR	DEN-2	146	6
Sotomayor-Bonilla J et al. (B)	2018	Mexico	Rodents	NR	NR	NR	NR	RT-PCR	DEN-2	708	0
Wolfe N et al.	2001	Malaysia	Non-human primates	PRNT	DEN-2	71	21	NR	NR	NR	NR
Diallo M et al.	2003	Senegal	Non-human primates	ELISA	DEN-2	17	10	NR	NR	NR	NR
Fagbami A et al.	1977	Nigeria	Non-human primates	PRNT	DEN-2	104	48	NR	NR	NR	NR
Dolz G et al.	2019	Costa Rica	Non-human primates	ELISA	DEN-2, DEN-3, DEN-4	8	3	RT-PCR	DEN-2, DEN-3, DEN-4	155	8
Inoue S et al.	2003	Philippines	Non-human primates	ELISA	DEN-1, DEN-2, DEN-3 and DEN-4	54	2	NR	NR	NR	NR
Peiris J et al.	1993	Sri Lanka	Non-human primates	PRNT	DEN-2	68	64	NR	NR	NR	NR
Rudnick A et al.	1965	Malaysia	Non-human primates	HI	DEN-2	114	94	NR	NR	NR	NR
			Pigs	HI	DEN-2	20	19	NR	NR	NR	NR

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Author	*Year-Publication*	*Country*	*Type of animal*	*Serological method*	*Dengue serotype*	*N*	*n(+)*	*Molecular method*	*Dengue serotype*	*N*	*n(+)*
Catenacci L et al.	2018	Brazil	Non-human primates	HI	DEN-1, DEN-2, DEN-3 and DEN-4	110	31	NR	NR	NR	NR
			Sloths	HI	DEN-1, DEN-2, DEN-3 and DEN-4	29	14	NR	NR	NR	NR
Moreira-Soto A et al.	2018	Brazil	Non-human primates	PRNT	DEN-1	48	2	NR	NR	NR	NR
Kato F et al.	2013	Philippines	Non-human primates	ELISA	DEN-1, DEN-2, DEN-3 and DEN-4	100	35	RT-PCR	DEN-2	7	2
Rosen L et al.	1958	Panama	Non-human primates	HI	DEN-1 and DEN-2	105	2	NR	NR	NR	NR
Hemme R et al.	2016	Puerto Rico	Non-human primates	Microneutra-lisation test (MNT)	DEN-1, DEN-2, DEN-3 and DEN-4	23	23	NR	NR	NR	NR
Yuwono J et al.	1984	Asian continent	Non-human primates	PRNT	DEN-1	428	146	NR	NR	NR	NR
Zhang H et al.	1998	China	Bats	ELISA	DEN-1, DEN-2, DEN-3 and DEN-4	20	16	RT-PCR	DEN-1, DEN-2, DEN-3 and DEN-4	56	23
Abundes-Gallegos J et al.	2018	Mexico	Bats	NR	NR	NR	NR	RT-PCR	DEN-2	16	8
Bittar C et al.	2018	Brazil	Bats	HI	DEN-1, DEN-2, DEN-3 and DEN-4	73	0	RT-PCR	DEN-1, DEN-2, DEN-3 and DEN-4	103	0
Cabrera-Romo S et al.	2016	Mexico	Bats	PRNT	DEN-2 and DEN-4	240	0	RT-PCR	DEN-2 and DEN-4	240	0
O?Connor J et al.	1955	Australia	Bats	Mouse protection test	DEN-1, DEN-2	28	4	NR	NR	NR	NR
Irving A et al.	2020	Singapore	Bats	Luciferase immune-precipitation system	DEN-2	106	14	NR	NR	NR	NR
Kaul H et al.	1976	India	Bats	HI	DEN-1, DEN-2 and DEN-3	91	1	NR	NR	NR	NR
Stone D et al.	2018	Grenada	Bats	PRNT	DEN-1, DEN-2, DEN-3 and DEN-4	50	0	NR	NR	NR	NR
Price J et al.	1978	Trinidad and Tobago	Bats	HI	DEN-2	857	126	NR	NR	NR	NR
Ramos B et al.	2017	Brazil	Birds	HI	DEN-1, DEN-2, DEN-3 and DEN-4	85	0	NR	NR	NR	NR

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Author	*Year-Publication*	*Country*	*Type of animal*	*Serological method*	*Dengue serotype*	*N*	*n(+)*	*Molecular method*	*Dengue serotype*	*N*	*n(+)*
Thongyuan S et al.	2017	Thailand	Dogs	NR	NR	NR	NR	RT-PCR	DEN-1, DEN-2, DEN-3 and DEN-4	1302	8
Kolman J et al.	1975	Czech Republic	Birds	HI	DEN-1, DEN-2, DEN-3 and DEN-4	280	0	NR	NR	NR	NR
Ghosh S et al.	1975	India	Birds	HI	DEN-1, DEN-2, DEN-3 and DEN-4	759	93	NR	NR	NR	NR
Kalimuddin M et al.	1982	India	Pigs	HI	DEN-2	404	180	NR	NR	NR	NR
Pauvolid-Corrêa A et al.	2014	Brazil	Sheep	ELISA	DEN-1, DEN-2, DEN-3 and DEN-4	238	0	NR	NR	NR	NR
			Horses	PRNT	DEN-1, DEN-2, DEN-3 and DEN-4	760	8	NR	NR	NR	NR
Mall M et al.	1995	India	Dogs	HI	DEN-2	104	16	NR	NR	NR	NR
			Pigs			170	20	NR	NR	NR	NR
			Horses			170	27	NR	NR	NR	NR
			Buffaloes			333	26	NR	NR	NR	NR
			Sheep			168	0	NR	NR	NR	NR
			Cattle			252	0	NR	NR	NR	NR
Albanese M et al.	1971	Italy	Sheep	HI	DEN-1	130	1	NR	NR	NR	NR
			Cattle		DEN-1	410	1	NR	NR	NR	NR
Okia M et al.	1971	Uganda	Birds	HI	DEN-1, DEN-2, DEN-3 and DEN-4	221	15	NR	NR	NR	NR
Darwish M et al.	1983	Pakistan	Buffaloes	HI	DEN-1	33	2	NR	NR	NR	NR
			Rodents			157	2	NR	NR	NR	NR
			Sheep			46	5	NR	NR	NR	NR
			Cattle			45	0	NR	NR	NR	NR
								NR	NR	NR	NR
Hussen M et al.	2020	Egypt	Camel	ELISA	DEN-1, DEN-2, DEN-3 and DEN-4	91	3	NR	NR	NR	NR
Contigiani M et al.	2000	Argentina	Non-human primates	HI	DEN-2	68	3	NR	NR	NR	NR
Valentine M et al.	2020	West Indies	Non-human primates	ELISA	NR	858	0	NR	NR	NR	NR
Loach T et al.	1983	India	Birds	HI	DEN-2	308	140	NR	NR	NR	NR

NR: Not Reported; RT-PCR: Reverse transcriptase–polymerase chain reaction; ELISA: Enzyme-Linked Immuno Assay; HI: Hemagglutination inhibition; PRNT: Plaque reduction neutralisation test.

Table 3 - Quality assessment of included studies.

	NEWCASTLE - OTTAWA QUALITY ASSESSMENT SCALE FOR CROSS-SECTIONAL STUDIES								
STUDY	*SELECTION*				*COMPARABILITY*	*OUTCOME*			
	Representativeness of the sample	*Sample size*	*Non-respondents*	*Ascertainment of the exposure (risk factor)*	*The subjects in different outcome groups are comparable based on the study design or analysis. Confounding factors are controlled. Maximum: ☆☆*	*Assessment of outcome*	*Statistical test*	*SCORE*	*Evidence quality*
Zavala R et al.	☆	☆	☆	☆	☆☆	☆	☆	8	Low Risk of Bias
De Thoisy et al.	☆	☆	☆	☆	☆☆	☆	☆	8	Low Risk of Bias
Aguilar-Setién A et al.	☆	☆	☆	☆	☆☆	☆	☆	8	Low Risk of Bias
Nakgoi K et al.	☆	☆	☆	☆	☆☆	☆	☆	8	Low Risk of Bias
Eastwood G et al.	☆	☆	☆	☆	☆☆	☆	☆	8	Low Risk of Bias
Calderon A et al.	☆	☆	☆	☆	☆☆	☆	☆	8	Low Risk of Bias
Kading R et al.	☆	☆	☆	☆	☆☆	☆	☆	8	Low Risk of Bias
Platt K et al.	☆	☆	☆	☆	☆☆	☆	☆	8	Low Risk of Bias
Machain-Williams et al.	☆	☆	☆	☆	☆	☆		6	High Risk of Bias
Kilbourn A et al.	☆	☆	☆	☆	☆☆	☆	☆	8	Low Risk of Bias
Vicente-Santos A et al.	☆	☆	☆	☆	☆	☆	☆	7	Low Risk of Bias
Kading R et al.	☆	☆	☆	☆	☆☆	☆	☆	8	Low Risk of Bias
De Silva A et al.	☆	☆	☆	☆	☆☆	☆	☆	8	Low Risk of Bias
Cigarroa-Toledo et al.	☆	☆	☆	☆	☆	☆	☆	7	Low Risk of Bias
Beck C et al.	☆	☆	☆	☆	☆	☆	☆	7	Low Risk of Bias
De Oliveira-Filho et al.	☆	☆	☆	☆	☆	☆	☆	7	Low Risk of Bias
Sotomayor-Bonilla et al. (A)	☆	☆	☆	☆	☆	☆	☆	7	Low Risk of Bias
Sotomayor-Bonilla J et al. (B)	☆	☆	☆	☆	☆	☆	☆	7	Low Risk of Bias

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	NEWCASTLE - OTTAWA QUALITY ASSESSMENT SCALE FOR CROSS-SECTIONAL STUDIES								
STUDY	*SELECTION*				*COMPARABILITY*	*OUTCOME*			
	Representativeness of the sample	*Sample size*	*Non-respondents*	*Ascertainment of the exposure (risk factor)*	*The subjects in different outcome groups are comparable based on the study design or analysis. Confounding factors are controlled. Maximum: ☆☆*	*Assessment of outcome*	*Statistical test*	*SCORE*	*Evidence quality*
Wolfe N et al.	☆	☆	☆	☆	☆	☆	☆	7	Low Risk of Bias
Diallo M et al.	☆	☆	☆	☆	☆	☆		6	High Risk of Bias
Fagbami A et al.	☆	☆	☆	☆	☆	☆	☆	7	Low Risk of Bias
Dolz G et al.	☆	☆	☆	☆	☆	☆		6	High Risk of Bias
Inoue S et al.	☆	☆	☆	☆	☆	☆	☆	7	Low Risk of Bias
Peiris J et al.	☆	☆	☆	☆	☆	☆	☆	7	Low Risk of Bias
Rudnick A et al.	☆	☆	☆	☆	☆	☆		6	High Risk of Bias
Catenacci L et al.	☆	☆	☆	☆	☆	☆	☆	7	Low Risk of Bias
Moreira-Soto A et al.	☆	☆	☆	☆	☆	☆	☆	8	Low Risk of Bias
Kato F et al.	☆	☆	☆	☆	☆	☆	☆	8	Low Risk of Bias
Rosen L et al.	☆	☆	☆	☆	☆	☆	☆	8	Low Risk of Bias
Hemme R et al.	☆	☆	☆	☆	☆	☆	☆	8	Low Risk of Bias
Yuwono J et al.	☆	☆	☆	☆	☆	☆	☆	8	Low Risk of Bias
Zhang H et al.	☆	☆	☆	☆	☆	☆	☆	8	Low Risk of Bias
Abundes-Gallegos J et al.	☆	☆	☆	☆	☆	☆	☆	8	Low Risk of Bias
Bittar C et al.	☆	☆	☆	☆	☆	☆	☆	8	Low Risk of Bias
Cabrera-Romo S et al.	☆	☆	☆	☆	☆	☆	☆	8	Low Risk of Bias
O?Connor J et al.	☆	☆	☆	☆	☆	☆		6	High Risk of Bias
Irving A et al.	☆	☆	☆	☆	☆☆	☆	☆	8	Low Risk of Bias
Kaul H et al.	☆	☆	☆	☆	☆☆	☆	☆	8	Low Risk of Bias
Stone D et al.	☆	☆	☆	☆	☆	☆		6	High Risk of Bias
Price J et al.	☆	☆	☆	☆	☆☆	☆	☆	8	Low Risk of Bias
Ramos B et al.	☆	☆	☆	☆	☆☆	☆	☆	8	Low Risk of Bias

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	NEWCASTLE - OTTAWA QUALITY ASSESSMENT SCALE FOR CROSS-SECTIONAL STUDIES								
STUDY	SELECTION				COMPARABILITY	OUTCOME			
	Representativeness of the sample	Sample size	Non-respondents	Ascertainment of the exposure (risk factor)	The subjects in different outcome groups are comparable based on the study design or analysis. Confounding factors are controlled. Maximum: ☆☆	Assessment of outcome	Statistical test	SCORE	Evidence quality
Thongyuan S et al.	☆	☆	☆	☆	☆☆	☆	☆	8	Low Risk of Bias
Kolman J et al.	☆	☆	☆	☆	☆☆	☆	☆	8	Low Risk of Bias
Ghosh S et al.	☆	☆	☆	☆	☆☆	☆	☆	8	Low Risk of Bias
Kalimuddin M et al.	☆	☆	☆	☆	☆☆	☆	☆	8	Low Risk of Bias
Pauvolid-Corrêa A et al.	☆	☆	☆	☆	☆☆	☆	☆	8	Low Risk of Bias
Mall M et al.	☆	☆	☆	☆	☆☆	☆	☆	8	Low Risk of Bias
Albanese M et al.	☆	☆	☆	☆	☆☆	☆	☆	8	Low Risk of Bias
Okia M et al.	☆	☆	☆	☆	☆☆	☆	☆	8	Low Risk of Bias
Darwish M et al.	☆	☆	☆	☆	☆☆	☆	☆	8	Low Risk of Bias
Hussen M et al.	☆	☆	☆	☆	☆☆	☆	☆	8	Low Risk of Bias
Contigiani M et al.	☆	☆	☆	☆	☆☆	☆	☆	8	Low Risk of Bias
Valentine M et al.	☆	☆	☆	☆	☆	☆	☆	7	Low Risk of Bias
Loach T et al.	☆	☆	☆	☆	☆	☆	☆	7	Low Risk of Bias

lence of 10.0% (95% CI: 3.0%-19.0%; I^2=97.25%) (Figure 5).

Molecular methods

The presence of dengue in bats according to molecular methods (RT-PCR) was evaluated in 9 studies (n=1335), with a seroprevalence of 6.0% (95% CI: 1.0%-13.0%; I^2=94.78%) (Figure 6).

Non-human primates (NHP)

Serological methods

The presence of dengue in NHP according to serological methods was evaluated in 23 studies (n=2946), with a seroprevalence of 29.0% (95% CI: 16.0%-44.0%; I^2=98.46%) (Figure 7). When subgroup analysis was performed according to the type of serological methods (Figure 8), it was found that NHP evaluated by PRNT, ELISA and HI had a prevalence of 32.0%, 25.0% and 19.0%. In the subgroup analysis, according to continents (Figure 9), it was found that the bats evaluated in America, Asia, and Africa had a prevalence of 18.0%, 40.0%, and 33.0%, respectively. In their sensitivity analysis, no decrease in their heterogeneity was found, with a prevalence of 25.0% (95% CI: 13.0%-39.0%; I^2=98.37%) (Figure 10).

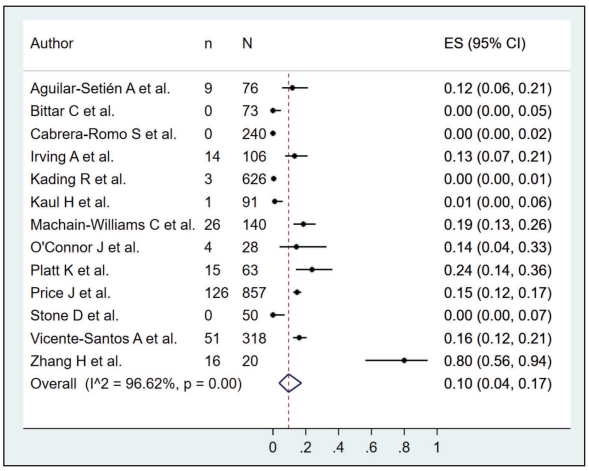

Figure 2 - Prevalence of dengue in bats according to serological method.

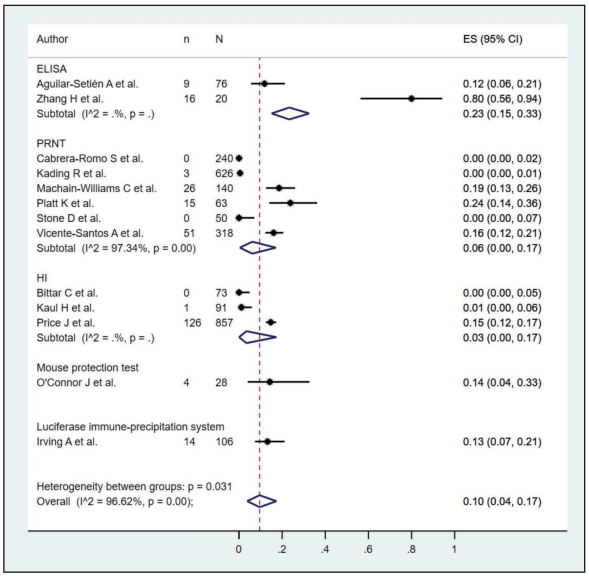

Figure 3 - Subgroup analysis according to type of serological method in the prevalence of dengue in bats.

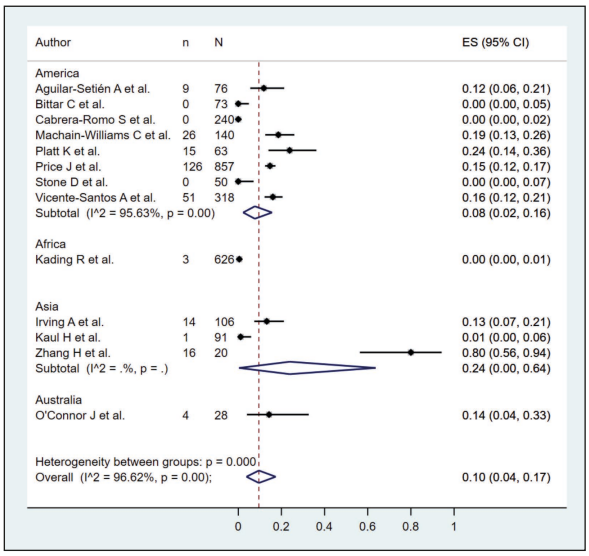

Figure 4 - Subgroup analysis according to continents in the prevalence of dengue in bats.

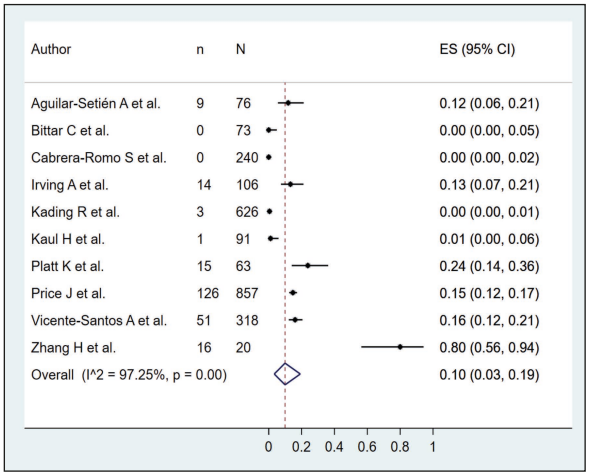

Figure 5 - Sensitivity analysis according to the risk of bias in the prevalence of dengue in bats.

Other animals

The presence of dengue in birds according to serological methods was evaluated in 5 studies (n=1653), with a seroprevalence of 8.0% (95% CI: 0.0%-25.0%; I^2=98.82%) (Figure 11). The occurrence of dengue in sheep by serological methods was evaluated in 4 studies (n=582), with a seroprevalence of 1.0% (95% CI: 0.0%-4.0%; I^2=82.42%) (Figure 12). In the case of horses, dengue was assessed by serological methods in 3 studies (n=1223), with a seroprevalence of 11.0% (95% CI: 0.0%-33.0%; I^2=98.7%) (Figure 13).

The presence of dengue in cattle by serological methods was evaluated in 3 studies (n=707), with a seroprevalence of 0.0% (Figure 14). The detection of dengue in pigs by serological methods was evaluated in 3 studies (n=594), with a seroprevalence of 49.0% (95% CI: 16.0%-83.0%; I^2=98.1%) (Figure 15). The identification of dengue in rodents by serological methods was evaluated in 3 studies

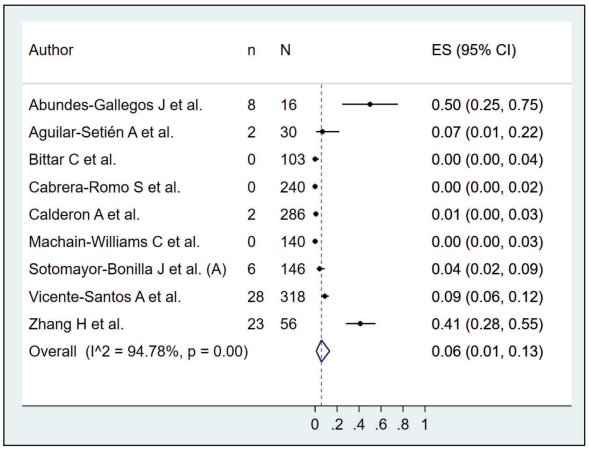

Figure 6 - Prevalence of dengue in bats according to molecular method.

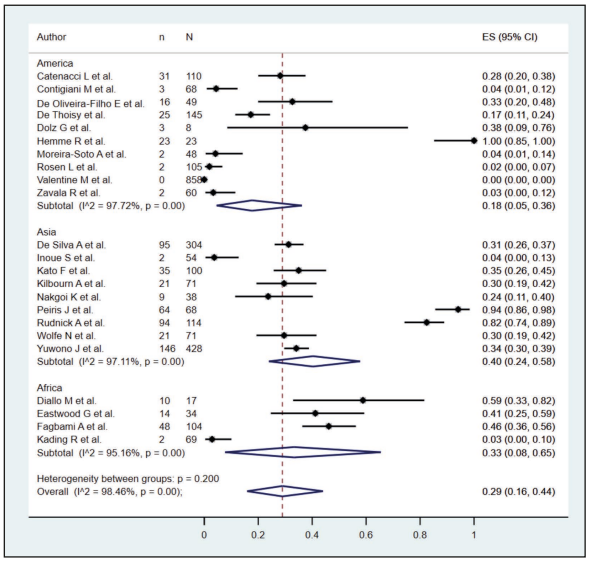

Figure 8 - Subgroup analysis according to type of serological method in the prevalence of NHP in bats.

(n=474), with a seroprevalence of 2.0% (95% CI: 1.0%-4.0%; I^2=98.1%) (Figure 16). Finally, the occurrence of dengue in buffaloes by serological methods was evaluated in 3 studies (n=390), with a seroprevalence of 7.0% (95% CI: 4.0%-10.0%; I^2=0%) (Figure 17).

DISCUSSION

During the last few years, an essential part of the research related to Arboviruses, such as dengue, has focused on better understanding the factors associated with its transmission, including understanding the animal reservoirs that could potentially serve as a natural source in the environment, from which transmission cycles can be established in wild and suburban areas, but perhaps also urban ones, using different urban and wild vector insects, of which *Aedes aegypti*, *Aedes albopictus*. *Aedes vittatus* has also stood out, recently reported

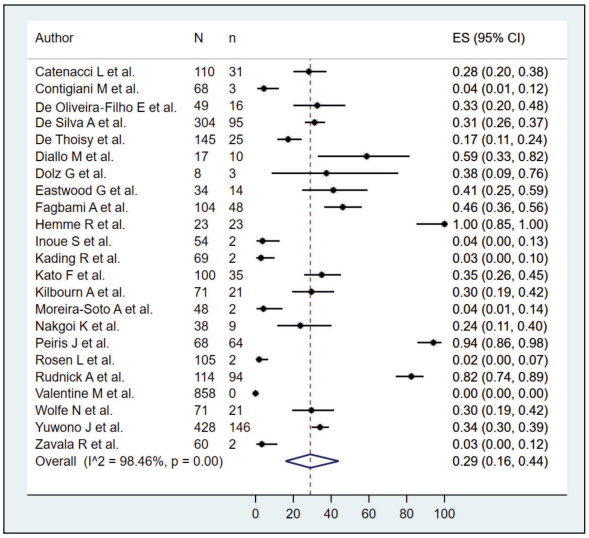

Figure 7 - Prevalence of dengue in non-human primates according to serological method

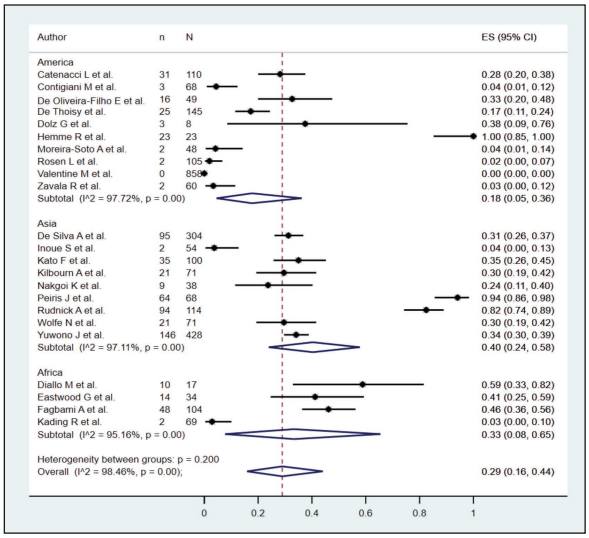

Figure 9 - Subgroup analysis according to continents in the prevalence of dengue in NHP.

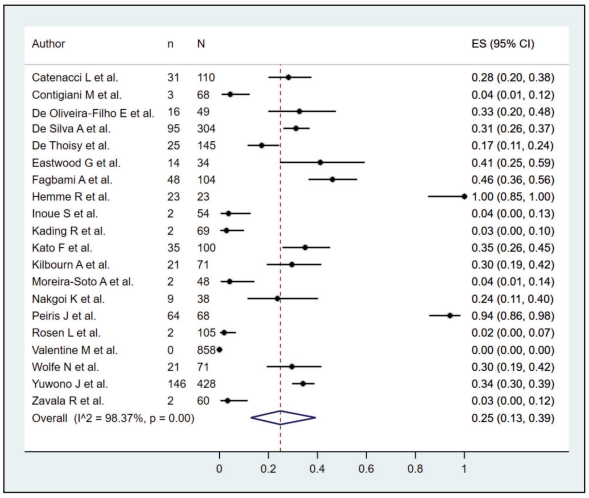

Figure 10 - Sensitivity analysis according to the risk of bias in the prevalence of dengue in NHP.

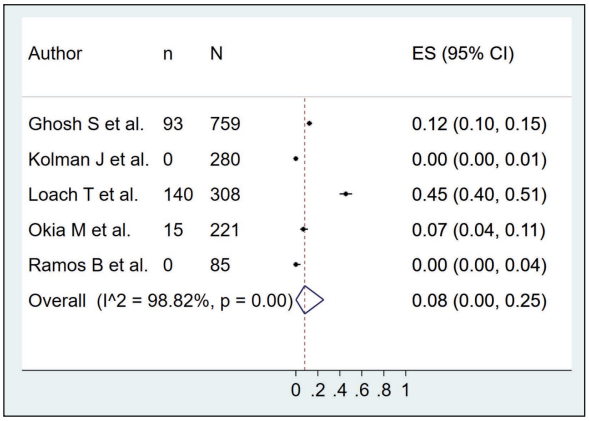

Figure 11 - Prevalence of dengue in birds according to serological method.

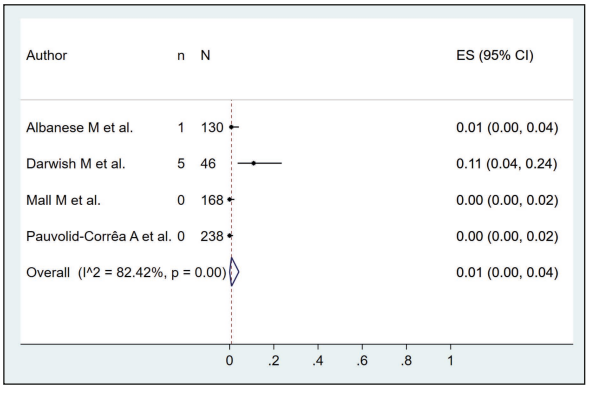

Figure 12 - Prevalence of dengue in sheeps according to serological method.

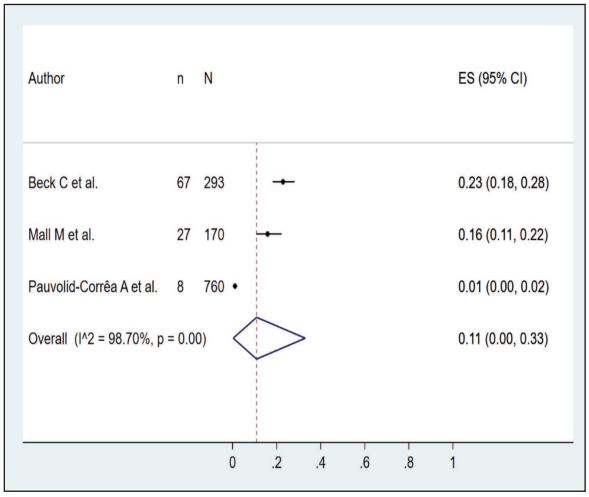

Figure 13 - Prevalence of dengue in horses according to serological method

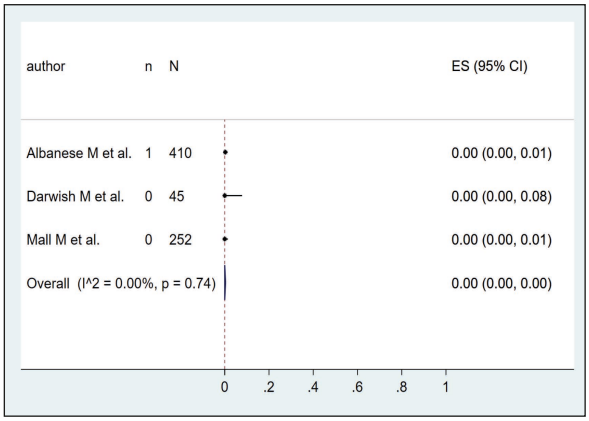

Figure 14 - Prevalence of dengue in cattle according to serological method

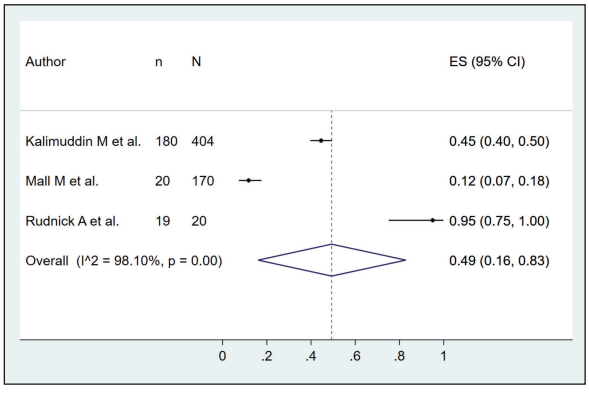

Figure 15 - Prevalence of dengue in pigs according to serological method.

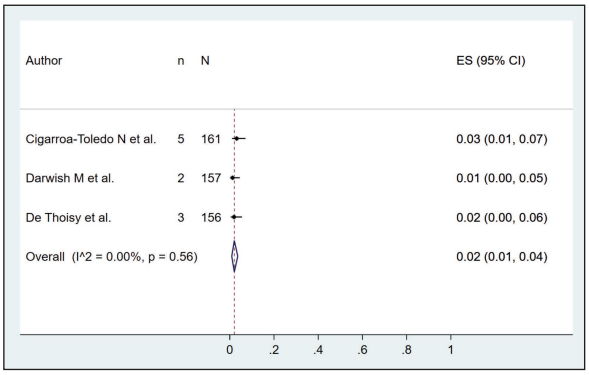

Figure 16 - Prevalence of dengue in rodents according to serological method.

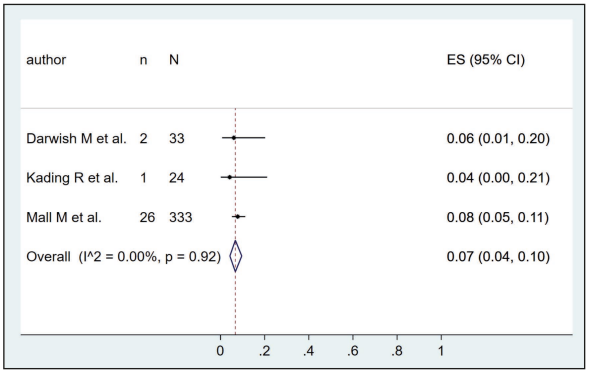

Figure 17 - Prevalence of dengue in buffaloes according to serological method

on the American continent. Dengue involves the sylvatic (enzootic) cycle and the endemic urban cycle, which involve non-human primates in sylvan habitats and humans in urban settings as reservoir hosts [72]. However, based on existing studies, not all primate species may be susceptible to Dengue virus infection [73].

Apart from non-human primates, bats are the most studied in detecting the presence of Dengue infection, and most studies include RT-PCR. As recently described in Colombia, in the department of Córdoba, with *Carollia perspicillata* and *Phyllostomus discolor*, two studies have confirmed the presence of Dengue virus in these bats [74]. In these studies, serotype 2 (DENV-2) has been identified with molecular diagnosis and sequencing.

In addition, other studies in Latin America, such as Mexico, found six bats (4.1%) positive for DENV-2 [75]. On the other hand, in Costa Rica and Ecuador, neutralising antibodies to dengue virus serotypes 1 and 2 and serotypes 2 and 3 were detected in 12 of 53 (22.6%) and 3 of 10 (30.0%) bats, suggesting that bats can be infected with the Dengue virus.

Bats are evolutionarily successful creatures widely distributed globally [76]. It is known that at least one species from each of the 19 families that make up the order Chiroptera perches in dwellings, which has implications for the bat-human closeness, which the presence of vector insects also surrounds, such as Aedes, which are not only anthropophilic but simultaneously zoophilic (amphiphilic) [77, 78].

In a previous systematic review covering up top 2019, Dengue positivity was detected in bats (10.1%), non-human primates (NHP) (27.3%), while in our study, 10.0% was also found in bats by serology, but was 24% in Asia, with 6% by molecular methods [77]. For NHP the seroprevalence was 29%, reaching 40% in Africa.

Relatively high DENV seroprevalence was also observed in marsupials, the primary reservoirs of the Ross River virus, a mosquito-borne Alphavirus [79]. In addition, dengue positivity was observed in birds, dogs, and rodents, animals commonly found in the urban environment. Based on the available evidence, it is not recommended to isolate animals in a general way. Phylogenetic studies would be interesting during epidemics, assessing the strains of dengue circulating in humans and animals, which may suggest transmission between them.

By extension, the abundance of these animals in urban settings potentially translates into a possible increased risk of exposure to dengue in humans who also become infected through mosquito bites. In addition, studies have also observed a wide range of hosts feeding on vector mosquitoes that efficiently transmit dengue, namely *Aedes albopictus* and *Aedes aegypti* and even now *Aedes vittatus*, present in the American continent [5, 80, 81].

The Dengue virus continues to represent a complex global public health problem, even worse in times of COVID-19 due to its cocirculation and coinfections, and in which a comprehensive, holistic vision of health is always required, where the role of the environment and animal health, can play a critical role. Veterinary research is needed to realise the importance of this last component [82]. In addition, they have begun to show the virus?s relationships and presence in these animals. Final-

ly, however, it is better to understand its weight in the different transmission cycles.

CONCLUSIONS

Several studies have shown that nucleic acids or antibodies to Dengue Virus (DENV) are present in Neotropical wildlife, including bats, suggesting that some species may be susceptible to DENV infection. Non-human primates have been widely used as models for studies on the pathogenesis of dengue and therapeutic interventions. However, they are also animals where the virus has been detected in natural conditions. Dengue virus can infect several animal species; however, its role as an amplifying reservoir is uncertain due to several limitations in the evidence. That suggests the need for more studies. In addition, the results lead to a greater need for more studies to evaluate the role in transmission, including assessing the feeding preference of the vectors. Given the relevant proportion of animals with positive results for dengue, surveillance in animals, especially during epidemics in humans, would be interesting, as in those endemic areas, as well as to increase surveillance in vectors, including studies of host preferences by *Aedes* species. Finally, they imply a greater need for studies in Colombia, an endemic disease country.

Conflict of interest
None.

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Ethical approval statement
Not applicable.

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