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Corresponding Author(s):	Kirstyn Brunker
Corresponding Author' Email:	kirstyn.brunker@glasgow.ac.uk
Corresponding Author's Department:	School of Biodiversity, One Health and Veterinary Medicine
Corresponding Author's Institution:	University of Glasgow
Country/Region:	United Kingdom
Order of Author (Only first three authors will be listed):	1
Author's Name:	Gurdeep Jaswant
Order of Author:	2
Author's Name:	Criselda Bautista
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Meta-Analysis/Systematic Review

Viral Sequencing to Inform The Global Elimination of Dog-Mediated Rabies - A Systematic Review

Gurdeep Jaswant[1-4]*, Criselda T. Bautista[2,5]*, Brian Ogoti[1,6], Joel Changalucha[4,7], Julius O. Oyugi[1,8], Kathryn Campbell[2], Mumbua Mutunga[1,6], S.M. Thumbi*[1,6,7,8,9,10], Katie Hampson*[2], Kirstyn Brunker*[2]

[1]Faculty of Health Sciences, Institute of Tropical and Infectious Diseases, University of Nairobi, Nairobi, 00202, Kenya.

[2]School of Biodiversity, One Health & Veterinary Medicine, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow, G12 8QQ, Scotland, UK.

[3]Food Biotechnology and Microbiology, Tanzania Industrial Research Development Organization, Dar es Salaam, 14111, Tanzania.

[4]Environmental Health and Ecological Sciences, Ifakara Health Institute, Dar es Salaam, Plot 463, Tanzania.

[5]Research Institute for Tropical Medicine, Muntinlupa City, Metro Manila, 1781, Philippines.

[6]Faculty of Health Sciences, Center for Epidemiological Modelling and Analysis, University of Nairobi, Nairobi, 00202, Kenya.

[7]College of Veterinary Medicine & Biomedical Science, Sokoine University of Agriculture, Morogoro, 67804, Tanzania.

[8]Department of Medical Microbiology, Faculty of Health Sciences, University of Nairobi, Nairobi, 00202, Kenya.

[9]Institute of Immunology & Infection Research, School of Biological Sciences, University of Edinburgh, Edinburgh, EH9 3FL, Scotland, UK.

[10]Paul G Allen School for Global Health, Washington State University, Pullman, 1155 NE College Ave, WA 99164, USA.

*These authors contributed equally to this work

Correspondence to: Kirstyn Brunker, School of Biodiversity, One Health & Veterinary Medicine, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow G12 8QQ, Scotland, United Kingdom

Email: kirstyn.brunker@glasgow.ac.uk

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Abstract

Background: Rabies is a fatal zoonotic disease, present in almost 150 countries. The 'Zero by 30' initiative aims to eliminate human deaths from dog-mediated rabies globally by 2030. This systematic review investigates how viral sequencing can contribute to achieving the 'Zero by 30' goal by improving understanding of viral circulation and the impact of rabies control measures.

Methods: A comprehensive search of bibliographic databases was conducted focusing on research on rabies from regions with endemic dog-mediated rabies published between 2000 and 2023, adhering to PRISMA guidelines. Data were extracted and synthesised to provide recommendations for further research and application to support rabies control.

Results: 220 studies were identified to have documented rabies virus sequences from 94 countries, primarily using first-generation technology to produce partial genomes and with sequencing predominantly conducted overseas rather than in-country. Dogs were identified to be the primary rabies virus reservoir in these regions, although some studies identified more localised wildlife reservoirs. Clade classifications were commonly based on host association or geographical location, however, lack of standardised methods and nomenclature for classifying lineages limited comparison at higher resolution. Cross-species transmission**,** and both local and long-distance transmission were identified, although quantitative inference was limited. Sequence data was particularly useful for identifying transboundary spread and incursions, investigating host shifts, and tracing sources of human rabies, with endemicity typically characterised by the identification of multiple co-circulating viral lineages.

Conclusion: There is an urgent need for standardised classification methods and phylogeny-based nomenclature for rabies viruses, and for improved sequencing capacity in regions with endemic dog-mediated rabies, including proficiency in bioinformatics and phylogenetics. Our findings emphasise the critical need to foster international cooperation and coordinate rabies control efforts to reduce transboundary spread, limit reintroductions and maintain progress towards the 2030 target.

Keywords: Rabies virus; genetic sequencing; phylogenetic analysis; rabies control and prevention; surveillance

INTRODUCTION

Rabies Virus (RABV) poses a major public health threat, causing around 60,000 deaths annually, almost exclusively in Low- and Middle-Income Countries (LMICs)[1]. The virus is most commonly transmitted through bites from infected hosts in the orders *Chiroptera* and *Carnivora*[2]. Domestic dogs are the main source of transmission to humans but, as a multi-host pathogen, wild carnivores also serve as primary RABV hosts with host-associated variants recorded in certain geographies[3]. For example, wildlife such as raccoons, skunks and foxes each maintain different RABV variants in localities across North America[3]. Generally, RABV is referred to according to these host-associated variants (sometimes termed biotype, see defined key terms in Box 1). Phylogenetic analysis enables further classification of RABV diversity into clades, subclades and lineages. The RABV genome is 12 kilobases (kb) in length[4], comprising five genes encoding the nucleoprotein (N), phosphoprotein (P), matrix protein (M), glycoprotein (G) and the large polymerase protein (L)[5]. Like other RNA viruses, RABV exhibits elevated mutation rates because of the absence of proofreading[6]. These mutation rates foster genetic diversity, facilitating tracking of viral spread and enhanced understanding of viral dynamics.

There is no treatment for rabies once clinical signs begin, but post-exposure prophylaxis (PEP) correctly administered shortly after exposure is almost 100% effective in preventing the fatal onset of disease[7]. However, a highly effective canine vaccine is available to prevent disease in the primary reservoir, and therefore prevent transmission to humans. Canine rabies elimination is possible through mass dog vaccination, as demonstrated in Europe, North America, and parts of Asia and Latin America[8]. Several countries where dog-mediated rabies was endemic have now been declared rabies-free or are approaching elimination as a result of sustained dog vaccination[8]. According to the World Health Organisation, to eliminate dog-mediated rabies vaccination campaigns need to achieve coverage of at least 70% and be conducted annually for at least three years[9]. Rabies incidence in Latin America has declined dramatically over recent decades due to coordinated regional dog vaccination programs[8]. In contrast, most LMICs in Asia and Africa have not allocated sufficient budget to control this disease, and access to dog vaccines remains limited. Confounding the lack of vaccines, typically, rabies surveillance has also been poor. Additional challenges to rabies control include lack of understanding of dog ownership patterns, population sizes, and accessibility for vaccination as well as cultural practices including dog meat consumption[10]. To address these challenges, international organisations joined forces under the United Against Rabies collaboration to advocate for the global goal of 'Zero by 30'; to end human deaths from dog-mediated rabies by 2030[11].

Surveillance plays a critical role in infectious disease control[12]. Surveillance entails the continuous, systematic collection, analysis, interpretation, and timely dissemination of health-

related information[13], serving as the foundation for planning, execution and evaluation of public health strategies. For instance, surveillance provides data on the effectiveness of interventions, supporting decision-making for initiatives like 'Zero by 30'[14]. Increasingly, surveillance also involves genetic data, for pathogen diagnosis and risk assessment, as well as to identify the source of outbreaks and to characterise pathogen spread[15]. Linked with locations, pathogen genetic data have uncovered disease movement; from global migration dynamics to local transmission pathways for pathogens such as Influenza virus[16,17], Ebola virus[18], Zika virus[19], Yellow fever virus[20,21], Mpox virus[22–25] and SARS-CoV-2[26]. Sequencing approaches have the potential to enhance rabies surveillance and provide actionable information to inform control programs locally and globally as part of 'Zero by 30'. For example, viral sequence data can distinguish undetected local circulation from incursions and potentially identify their sources[27]. More generally, sequencing could provide insights into how rabies circulates within populations and the processes responsible for its maintenance in specific localities[15,28].

Use of pathogen sequence data for surveillance is, however, not yet routine in most LMICs. Constraints include lack of local sequencing capacity, competent personnel and laboratory resources, and these are affected by costs of, and access to, reagents and consumables, as well as power supplies and cold chain[18]. Sequencing technologies have become more affordable and efforts are underway to improve accessibility[29]. Indeed, growth in sequencing capacity during the COVID-19 pandemic provided evidence of the feasibility of scaling up molecular diagnostics, but also highlighted operational challenges. For example, in Nigeria, the number of laboratories capable of molecular identification of SARS-CoV-2 increased from four to 72 in 2020[30]. In this systematic review, our goal was to examine the extent of the application of genetic approaches to RABV surveillance in regions with endemic dog-mediated rabies (much of Africa, Asia, and parts of Latin America) and how, going forward, these approaches can contribute to the 'Zero by 30' goal.

Box 1. Definitions of key terms in the context of RABV

Cross-species transmission: Transmission events from one (host) species to another, that occasionally result in a *host shift*, whereby a new transmission cycle is established, but more frequently leading to short-lived chains of transmission or dead-end infections with no onward transmission[31,32].

Reservoir: One or more epidemiologically connected populations in which the pathogen persists and from which infection is transmitted to a population of concern i.e. a *target* population[33]. Domestic dogs are considered *maintenance hosts* in the reservoir for rabies in

many regions, while humans, endangered wildlife, and livestock are often considered *target* populations[34].

Variant: A viral population maintained within a particular reservoir host in a geographically defined area that differs from other viral populations due to either a *host shift* or diversification within a host species or population ie. *host-association*[35] (sometimes called a biotype[36]). RABV variants often show *host-associations*. Here we differentiate dog- vs wildlife-associated RABV variants[37].

Directionality of transmission: The predominant direction of transmission from one host species, population or location to another. Genomic data can be used to identify how infected hosts are linked to each other, and to infer the source of infection[38]. *Transmission networks* involve mapping transmission routes or pathways to ascertain who infected whom and have been inferred for RABV using parsimony-based approaches and advanced Bayesian frameworks[38].

Transboundary spread: pathogen spread across administrative boundaries. We differentiate *human-mediated* spread, often over long distances e.g. >50km, from *local dispersal* due to host behaviour (rabid dogs typically bite animals within 1 km of their location, but can sometimes run over 20 km)[39]. An **incursion** (or introduction) is the spread of a pathogen into a new area, either where that pathogen was historically absent, had been previously eliminated (i.e. *re-introduction*), or where the pathogen is already present. In the latter example, an incursion might be identified from genetic data when a distinct viral lineage is found in an area where other lineages are circulating[40,41].

Phylogeny or phylogenetic tree: A branching diagram or tree showing the evolutionary relationships between sequences or species. These generally incorporate nucleotide substitution models, and can include taxonomic and temporal information. The most widely applied methods for tree building are Neighbour Joining, Maximum Parsimony, Maximum Likelihood and Bayesian Inference.

RABV - Gene-linked by Underlying Evolution (RABV-GLUE)[42] is a flexible software system for interpreting sequencing data with functionality for storage and interpretation. The software is freely available and can be directly downloaded for viral sequence analysis or can be used via the web interface: http://rabv-glue.cvr.gla.ac.uk.

Clade: A monophyletic group with a single common ancestor[43]. Shared ancestry therefore defines the initial pathogen emergence and spread[44]. RABV is designated into clades or

subclades (sometimes referred to as major and minor clades), usually associated with specific geographic areas and/or hosts. A ***lineage*** (sometimes referred to as a subtype) is a group of related sequences (typically a smaller monophyletic group contained within a larger subclade) defined by statistical support of their phylogenetic placement and genetic differences from their *most recent common ancestor* (mrca)[45]. Clades and subclades can be classified differently depending on the hosts and geographical location. In this review, publications may have designated clades/subclades/lineages according to the author's naming system. To allow for comparison between publications, a table of publication-specific names and corresponding RABV-GLUE designations is available in Supplementary Table 1.

Phylodynamics: The study of how epidemiological, immunological, and evolutionary processes (inter)act to shape viral phylogenies. Examples include studies of spatial diffusion, sometimes incorporating geographical or population structure i.e. *phylogeography*[46].

Genomic/ genetic surveillance: Surveillance involving sequence data to characterise an infectious agent and infer additional information about its dynamics. Genomic refers specifically to *Whole Genome Sequencing (WGS)* i.e. the entire pathogen genome (>10kb for RABV), whereas *partial genome sequencing* is the generation of a sequence of a specified genetic region that can be used to identify the organism. For RABV, 400 bp fragments are used for diagnostics, and many phylogenetic studies sequence the N gene, G gene and/or G-L intergenic regions.

1st generation (or Sanger) sequencing: Type of sequencing which produces DNA fragments labelled by chemical modified nucleotides (dideoxynucleotides) during nucleotide elongation. Sanger sequencing sequences one fragment at a time[47].

Next generation sequencing (NGS): Parallel approaches that sequence millions of fragments simultaneously, hence have higher throughput than 1st generation approaches. NGS technologies are divided into *2nd generation* technologies which analyse clonal representations of the input DNA before sequencing amplified DNA clones, e.g. Illumina MiSeq and Ion Torrent, versus *3rd generation* single molecule sequencing technologies, which can produce longer reads than 2nd generation platforms but typically have higher error rates, e.g. Oxford Nanopore and PacBio[47].

METHODS

This review was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines[48], following a protocol developed *a priori* to ensure methodological reproducibility and transparency (Supplementary File 1).

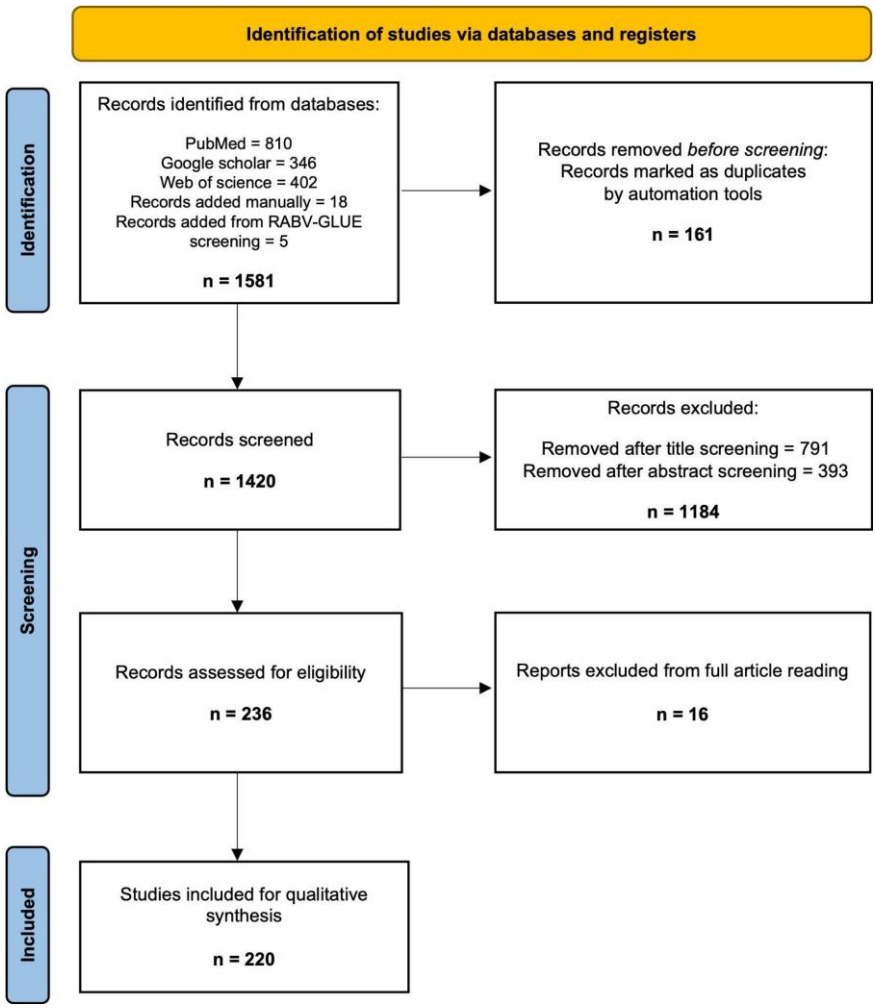

Figure 1. Flow diagram of the article selection process following PRISMA guidelines.

Eligibility criteria: Studies must address either dog, human or terrestrial wildlife rabies (i.e. not focus on bat rabies), and use molecular techniques and genetic sequence data, either for diagnosis (diagnostic PCR products) or surveillance. We excluded studies reported as literature reviews that did not present genetic sequence data, or focus on rabies, and that were not published in English.

Search strategy: A systematic search was undertaken on PubMed, Web of Science and Google Scholar databases to identify original studies published between 2000 and 2023. Advanced searches with Boolean operators and quotations were performed using the search terms “Rabies AND (genom* OR sequenc* OR molecular OR phylo*) AND (control OR surveillance OR

eliminat*)" as illustrated in supplementary Table 2 with an example of the medical subject headings (MeSH) terms from one of the search engines provided in supplementary Table 3 . Further manual searches were performed for additional relevant studies.

Screening: We reviewed the title and abstracts of all articles that met the inclusion criteria. Where detail was lacking, the entire article was reviewed before its inclusion or exclusion was determined. A first screening phase based on titles and abstracts was conducted independently by three independent reviewers (GJ, CB and MM), and out-of-topic studies excluded. During this phase, studies that were not covering rabies from Asia, Africa or Latin America were also excluded. A second screening phase based on the full texts was conducted by GJ and CB using a standardised eligibility form. Discrepancies observed between reviewers were resolved through discussion. Duplicate studies were removed.

Data extraction: A form designed for this review was used for data extraction (Supplementary Table 4). The fields extracted included: authors, year of publication, country, study aim, study design, species from which samples were collected, numbers of samples (tested and confirmed for rabies and RABV sequences), sample type, sequence type (WGS or partial genome, indicating the length and section sequenced), sequencing platform, type of phylogenetic analysis, outcome of the study, and the main study findings, including any recommendations for control measures derived from analysis of sequencing data.

Data synthesis: The main characteristics of the studies were summarised in tabular form (as per the data extraction proforma). Data analysis and visualisation was carried out in R (version 4.0.3)[49].

RESULTS

Study selection

The database search identified 1558 publications, of which 161 were excluded as duplicates using automation software[50]. Manual searches identified an additional 23 relevant publications. After screening and assessing eligibility, 220 were retained for systematic review (Figure 1).

Study Characteristics

The 220 articles generated new RABV sequences from 94 countries (Figure 2), with most from China (n=54 publications) and Brazil (n=19) while six undertook large-scale meta-analyses with additional sequencing from multiple countries[51–56]. An average of two studies presenting new RABV sequences were published per year (Figure 2), with most in 2013 (n=16) and 2015 (n=17).

All studies generated RABV sequences from brain tissue samples, with some on FTA cards[4,57–59] and four including alternative sample types (nuchal biopsy, cerebrospinal fluid and salivary glands). Most publications (n=188) reported results from partial sequences only, using 1st generation sequencing, mostly the N gene (n=119). Other studies sequenced the G, P or M genes or the G-L intergenic region, and 54 were multi-gene (Figure 3). Twenty-nine studies generated WGS, with hotspots in China (n=10) and Tanzania (n=5), and nine used multiple platforms (1st and 2nd[42,52,60–63] or 1st and 3rd generation platforms[64,65]). In the last decade, 3rd generation sequencing (Nanopore) increased, as did sequencing output.

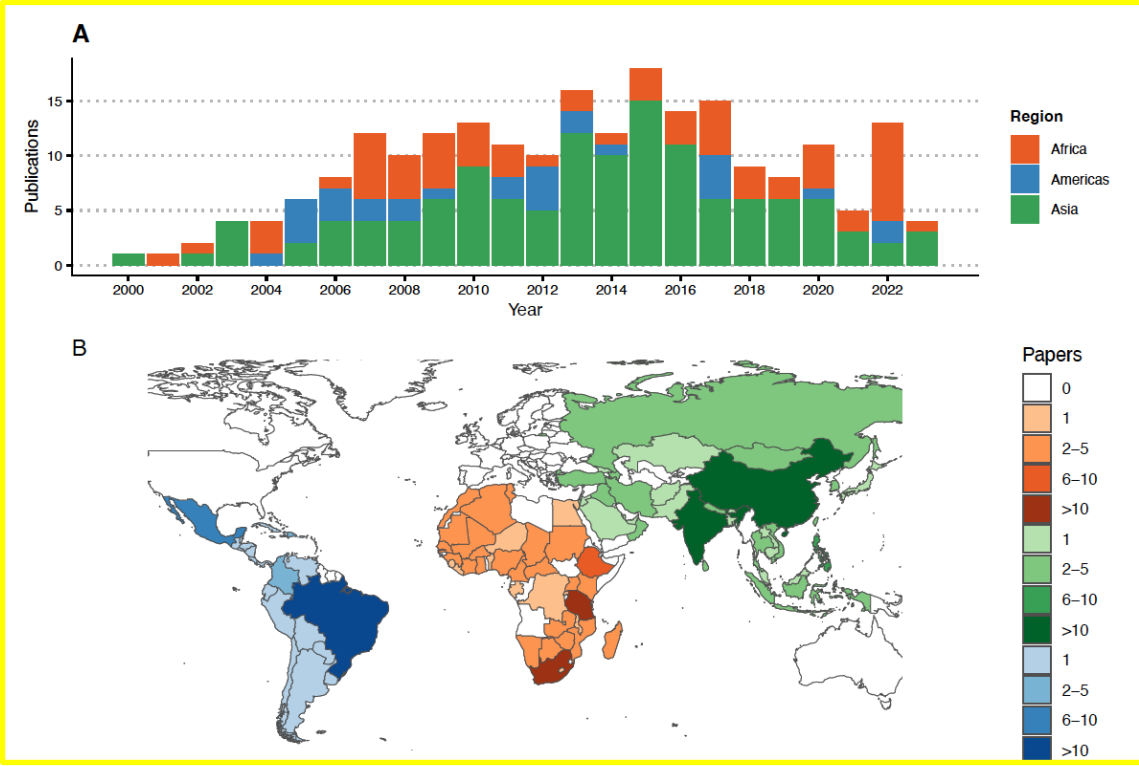

Figure 2. Publications reporting RABV sequences by region from 2000 to 2023. A) Time series of publications. B) Numbers of publications reporting sequence data by country, shaded by the number of publications and coloured by geographic region. Each publication was attributed to one or multiple countries based on the origin of the RABV sequences, including five studies reporting travel-associated human cases according to the country of origin. One study describing the global distribution of lyssaviruses[52], was excluded from this figure.

Sequences were generated from 94 of 149 countries in endemic regions (61.7%). Among the endemic regions, countries in Africa conducted the least in-country sequencing (42.8%, 14/27) whereas countries in Asia conducted the most (76.6%, 23/30, Figure 3). In terms of sequencing output, Asia led with 6,715 sequences (381 WGS), followed by Africa with 3,757 sequences (315 WGS) then Latin America with 1,143 sequences (26 WGS). Tree-building methods used for analyses in publications included Neighbour Joining (n=76), Maximum Likelihood (n=63),

Bayesian Inference (n=27) and a combination of these methods (n=48). The main study objectives reported were to identify circulating RABV, describe transmission dynamics, including the species infected and responsible for circulation, and to report viral movement. More generally, studies aimed to derive recommendations to inform control.

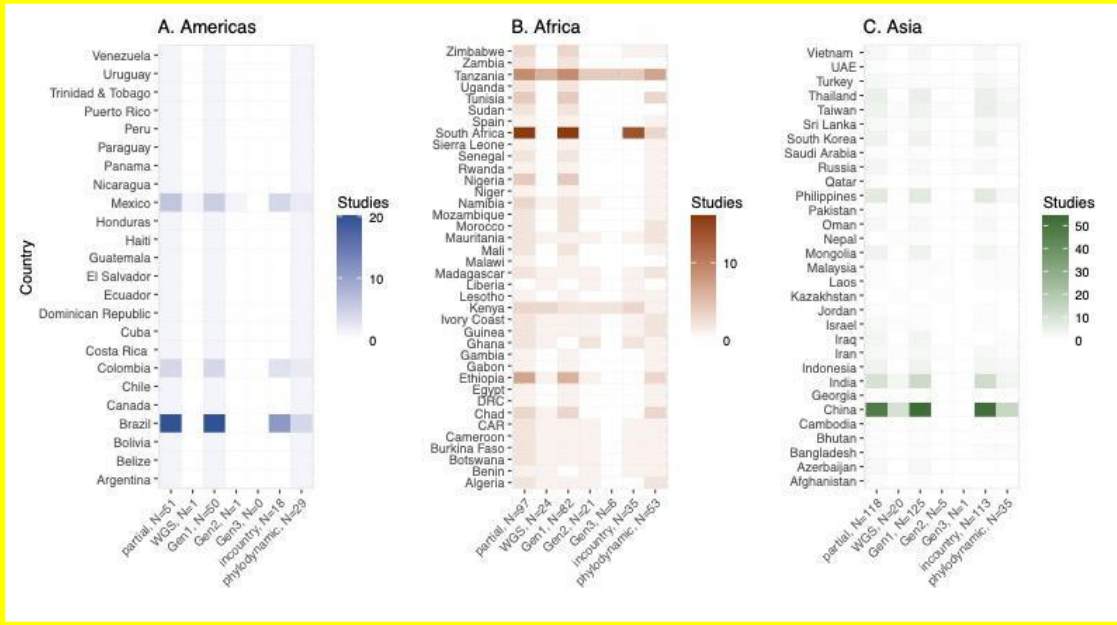

Figure 3. Summary of RABV sequencing by country, including the sequencing platform, type of sequence generated, location of sequencing and analyses undertaken. The colour intensity increases with the number of publications per country; note the different colour scale by regions; A) Americas, B) Africa, C) Asia. Only twenty nine publications generated WGS (from 4 countries) and nine conducted phylogeographic analyses. Three publications present data from across multiple countries, with two of these increasing both the number of countries with WGS and phylogeographic analyses[51,52]. An enlarged version of this figure is available as Supplementary Figure 1

Circulating RABV

A large proportion of the 220 studies reviewed described the diversity and distribution of circulating RABVs. Broadly, RABV can be classified into dog-related and bat-related viruses, both of which split into major clades, subclades and lineages[42,52], often associated with geographies and hosts. Publications from Latin America typically differentiated dog-related rabies versus bat-related rabies[56,60,61], and dog-derived and bat-derived versus skunk variants[56]. In Africa and Asia, RABV diversity was classified predominantly by geographical clustering of dog-related viruses. Generally, there was a lack of standardisation beyond global clade nomenclature, with many studies (n=92) introducing ad hoc names to refer to diversity within global clade assignments. Figure 4 uses RABV-GLUE[42] designations to depict circulating clades and a key indicates alternative names used in publications (Supplementary Table 1).

Three RABV clades are reported to circulate in Africa; the ‘Cosmopolitan’, ‘Africa 2’ (AF2) and

'Africa 3' (AF3), that subdivide into subclades and lineages[52]. The Cosmopolitan clade was found across 27 African countries and split into AF1a, AF1b, AF1c and AF4 subclades. AF1a was broadly distributed across the continent, and predominant in northern and eastern Africa. AF1b was mainly in eastern and southern Africa, while AF1c and AF4 were found in Madagascar and Egypt respectively[51]. The AF2 clade is found in 14 countries, mainly across West and Central Africa. The AF3 clade, which is associated with viverrids, is found in Southern Africa, with sequences from South Africa and Botswana[52] (Figure 4). In Asia, RABVs were categorised into four major clades: Cosmopolitan, Arctic (specifically the Arctic-like RABV, which has been found circulating across eastern and southern Asia), Indian Subcontinent, and Asian. The Cosmopolitan clade was widespread in Western[57,63–66], Eastern[67–75], and Northern Asia[76]. Five subclades of the Asian clade (SEA1-SEA5) were found within Eastern and Southeast Asia[77–80]. The Indian Subcontinent clade was prevalent in South Asia[81–84], with a few sequences from Western and Eastern Asia. While the Arctic-like clade was found in parts of Eastern[68,85–89] and South Asia[4,58,81,90–95]. In the Americas clades were categorised into dog-maintained and dog-derived variants, including established wildlife foci in skunks, coyotes, gray foxes and mongoose[56].

Concurrent circulation of divergent clades and subclades within countries, and more locally, was commonly reported across Asia and Africa. This was a less common feature in Latin America, where there are fewer remaining dog-mediated rabies foci and, overall, less dog-related RABV sequence data (almost all partial and just 26 WGS). Six African countries reported co-circulation of the Cosmopolitan and AF2 clades (Cameroon, the Central African Republic, Chad, Ghana, Gabon and Nigeria)[40,41,51,52,96,97]. One study identified co-circulation of Cosmopolitan AF1b subclade and AF3 clade in Botswana, but from different parts of the country; AF1b in the north and AF3 in the south[63]. Similarly six African countries reported co-circulation of Cosmopolitan AF1a and AF1b subclades (Cameroon, Chad, Ghana, Mozambique, Kenya and Uganda)[40,96,98–101]. All of the four major clades found in Asia were seen co-circulating within and between provinces in China[72–75,102–105]. In Southeast Asian countries, only the Asian clade was seen, except in Vietnam where the Cosmopolitan clade was also reported[80]. More differentiated subclades were reported from archipelagic Southeast Asian countries such as Indonesia (Asian SEA1b)[106–108], the Philippines (SEA4), Sri Lanka (Indian-Sub)[82,83], and Taiwan (SEA5)[109–111], with geographically-associated names used. As geographic resolution increased, naming systems became more ad hoc. For example, a study from the Philippines identified nine lineages (GrL1-9) within Group L of SEA4 subclade, named because of circulation within Luzon Island[112]

Many studies used nomenclature only meaningful to that study or research group. For example, sequences from China were variously referred to as groups I-IV[113,114], and lineages A-F[73,115]. The

same nomenclature could represent different diversity across studies. For example, group I referred to SEA1b sequences in one study[113], but SEA2a in another[114]. Another study defined 'Indian' lineages I & II[81], which were re-classified by RABV-GLUE (Arctic NA (i.e. no minor clade assigned), Arctic AL1a, Asian SEA1a, Indian-sub) revealing much broader diversity and geographic distribution than user-defined terminology implied. In Latin America, most studies (n=29) grouped RABVs into lineages named by geographical location or species involved, without following a common nomenclature.

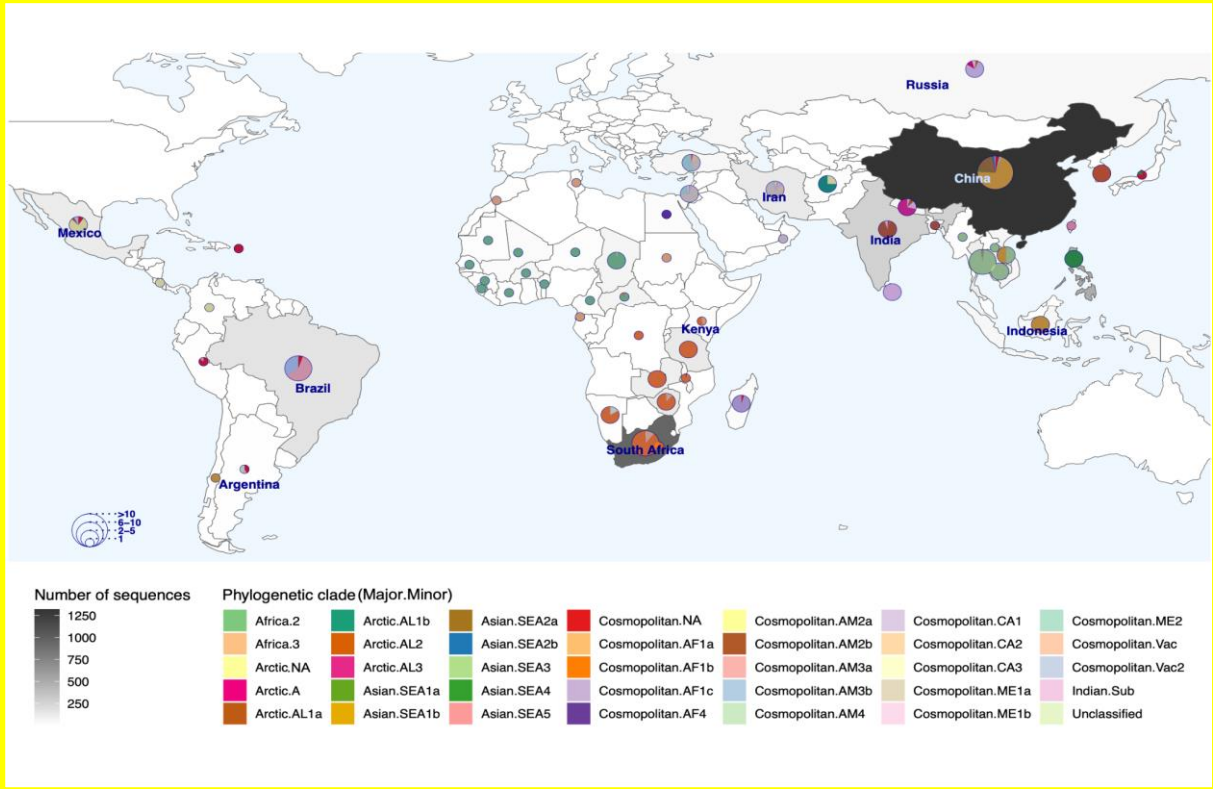

Figure 4. The spatial distribution of dog-associated rabies virus (RABV) clades reported from Asia, Africa and Latin America. Each country is shaded according to the number of publicly available sequences with at least 90% gene or genome coverage and labelled with a pie chart representing the RABV phylogenetic clades (denoted as Major.Minor clade in the legend e.g. Cosmopolitan.AF1a is major clade Cosmopolitan and minor clade AF1b, while sequences lacking resolution for a minor clade designation are assigned 'NA') within each country and sized according to the number of publications. Note that major and minor clades are annotated according to RABV-GLUE designations[42], but may be annotated differently in primary publications. The correspondence between annotations are detailed in Supplementary Table 1.

Host species

Studies reported RABVs from a broad range of domestic and wildlife hosts and humans. Sequences from domestic animals included dogs, cats, cows, goats, sheep, pigs, camels and horses. RABVs were found in wild carnivores such as foxes, jackals, hyenas, mongoose, African wild dogs, wolves, ferret badgers, and civets as well as occasionally in herbivores (e.g. nilgai and kudu) and more unusual wildlife like monkeys and bears. Eighty-five publications reported sequences from across this species range, 19 from just dogs and livestock, 18 from dogs and wildlife, and 15 from dogs and humans. Cross-species transmission, resulting in spillover, were reported in 63.7% (n=137) of studies: 13.1% (n=18/137) reporting transmission between domestic dogs and wildlife, and 86.9% (n=119) reporting transmission from dogs to both wildlife and livestock. In regions with endemic dog-mediated rabies most livestock outbreaks were attributed to spillover from dogs, except for outbreaks in livestock in Latin America from vampire bat rabies. However, spillover from wildlife to both dogs and to livestock was also observed, as seen in a livestock outbreak linked to foxes in northwest China[72]. Atypical cases, such as rabies in a chicken bitten by a dog, also highlight spillover events in endemic areas[84].

Domestic dogs were predominantly identified as the reservoir of RABVs within the defined scope of this study. Successful control of dog-mediated rabies across Latin America has left bats as the main RABV reservoir, but emergence of independent wildlife-associated cycles were attributed to spillovers from bat, arctic fox and canine rabies[116]. For example, RABVs were associated with skunks in north-central Mexico, with coyotes in west-central Mexico, gray foxes in Colombia, crab-eating foxes in Brazil and mongoose in Caribbean islands[56]. Two publications from Africa reported other reservoirs: horizontal transmission of a canid variant (AF1b) in kudu in Namibia[117], and the distinct AF3 clade circulating in yellow mongoose in Southern Africa[62]. Evolutionary rates inferred from AF3 were outliers from dog-related clades, suggesting host-specific adaptation[52]. In Asia, a unique ferret badger variant was found in Taiwan, distinct from other Asian subclades[118,119].

Studies examined reservoir dynamics, investigating new variants and quantifying transmission within and between species. For example, an Israeli study showed emergence of a dog-related clade that was distinct from fox-associated clades that were predominant before fox-targeted oral rabies vaccination began. The emerging dog-related clade might have indicated a host shift, but sequence similarity to Turkish dog isolates suggested cross-border introduction as the cause of the clade emergence[120]. Bayesian phylogenetic inference from WGS of samples from Turkey spanning from 1999 to 2015 was used to refine the date of a host shift that did occur from dogs to foxes to ~1997[121]. A European red fox-associated variant reported from hyenas in Tanzania[122],

was concluded to stem from laboratory contamination as other studies found that sequences from both wildlife and dogs all belonged to typical and closely-related dog-mediated variants belonging to clade AF1b[35]. Further analysis confirmed the central role of domestic dogs in maintaining circulation of AF1b in this region[123]. In the same setting, sequencing an African civet cat case that was presumed to be spillover of dog-mediated rabies, also unexpectedly identified a novel highly divergent lyssavirus[124], and no cases of this variant have been found in dogs. The majority of spillover examples identified were from dogs into wildlife, but a limited number of publications reported spillover from wildlife to dogs or other animals, for example, from Coyote into dogs in Mexico[56].

Human rabies

In total 24 studies (16 from Asia, 6 from Africa and 2 from Latin America) reported sequences from human rabies cases, with a total of 219 human samples sequenced. Most of these studies (n=19) identified human cases to be the result of dog-mediated rabies only. Three studies reported wildlife exposure only including 1 racoon exposure from Nepal[66] and 2 fox exposures, one from India[125] and one from Mexico[126]. A longitudinal study from South Africa reported a majority of human cases from dog-mediated rabies as well as three cases spread from mongoose (AF3)[128]. One of the Latin America publications reported a veterinarian who became infected while handling rabid livestock that was determined through genetic analysis to be vampire bat rabies[129].

Several of the human cases occurred in rabies-free countries and were resolved through phylogenetic analysis. Eight were traced to immigrants with exposure histories in countries with endemic dog-mediated rabies where PEP was not received before travel to the (rabies-free) country of diagnosis. Analysis of the patient isolates revealed similarity with cases in postulated countries of origin, including a case in the UK imported from Nigeria[130] and a case in France imported from Mali[131], two unrelated cases in Qatar both imported from Nepal[66], and cases imported from the Philippines to the UK[132], Japan[133] and Finland[134], as well one that likely originated from insectivorous bats involving a Mexican immigrant who was bitten by a fox, and who died in California[126]. Similarly a sequence from a patient in France, with recent travel history to Mali but no known exposure, belonged to AF2 subclade that circulates in West Africa[131].

Human cases with prolonged incubation periods were also identified (normal incubation periods range from 1 week to 8 months). One human case in rabies-free Australia was traced to exposure before relocation. The 10-year-old of Vietnamese origin had stayed in Hong Kong before immigrating to Australia 5 years prior to symptoms onset. Phylogenetic analysis revealed the isolate's distinctness from Australian Bat Lyssavirus and Vietnamese RABV lineages; instead grouping with a China-associated lineage, suggesting acquisition in Hong Kong and prolonged

incubation[135]. Two human cases imported to Japan also had incubation periods exceeding 8 months[133]. Most human cases resulted from bites, with two exceptions in Africa where victims were scratched or licked[136,137]. The first case involved a 26-month old child scratched by a puppy in Gauteng Province, South Africa. Genetic analysis linked this case to an outbreak that spread in dogs in southern Johannesburg following an introduction from KwaZulu-Natal[137]. The second case, a 6-year-old from KwaZulu-Natal, had no bite history, however his neighbour's dog died of unknown causes around the same time and phylogenetic similarity of sequences from the neighbours' dog and the child suggested the child was exposed from the neighbour's dog[129].

Rabies virus movement

Phylogeographic analysis identified local transmission and long-distance movement of rabies viruses based on the geographic association or displacement of clades, subclades and lineages. Some studies revealed situations characterised by sustained local circulation, with distinct lineages and closely related sequences confined within specific geographic areas. For instance, in Nigeria, sequences within AF2 (AF2-1 and AF2-2) clustered in Northern and Southern Nigeria respectively[130]. Similar clustering was observed in India[81,94] and major island groups in the Philippines[78,138]. However, local circulation was often not confined by political borders across contiguous landscapes. For example, samples from the border between Brazil (Mato Grosso do Sul state) and Bolivia clustered, reflecting frequent movement across this boundary [139].

Most publications mentioned human-mediated long-distance viral movement but only about 25% substantiated their claims through phylogeographic analysis. In Tanzania, discrete phylogeographic analysis revealed long-distance movement between regions (>750 km apart)[140], and sequences from distant ecosystems (Serengeti and Tarangire, >200 km apart) grouped together[35], with seasonal migration of pastoralists (and their dogs) proposed as an explanation. In Asia, long-distance movement was attributed to waves of human migration from China to Southeast Asia[141]. Indeed, increased trade between and within countries[142], including animal trade [75,104]and the dog meat trade[77,113,143], were identified as playing a role in RABV incursions and expansion.

Three sources of evidence were used to infer transboundary spread: co-circulation of divergent RABVs, clustering of related sequences from adjacent countries/regions and phylogeographic analyses using location data associated with sequences. Studies reported co-circulating divergent viruses in 31 countries, likely caused by introductions that persisted[40,41,96,99,100,144]. For example, AF1a, AF1b and AF2 were found co-circulating in southern Cameroon, with the AF1b and AF2 sequences most closely related to sequences from the Central African Republic[96]. RABVs circulating in neighbouring countries were often closely related, particularly across shared land

borders, like Azerbaijan[57] with Georgia, Bangladesh[90] with India, and Tibet[142] with Nepal. There were also instances of emerging subclades within one country closely connected to another. For example, Nepalese isolates identified as a new lineage within the Arctic-like (AL-1) subclade that circulates in India[4]. Indeed, some studies aimed to quantify transmission between countries[74,102]. One study highlighted the importance of China, suggesting the country was a source of translocation to 12 Asian countries due to migration and trade[68]. Similarly another study used Bayesian inference to test the hypothesis that AF2 was introduced to Ghana from other West African countries with results suggesting spread from Nigeria[99].

Several studies used phylogeographic analyses to infer RABV spatiotemporal dynamics[74,79,111,141,145], particularly the direction and speed of dispersal[45,68,146,147]. Faster dispersal was associated with anthropogenic factors. Dog-associated lineages in Brazil dispersed at 30.5 kilometres per year (km/yr) in comparison to a lower rate, 9.5 km/yr, in crab-eating foxes, which was attributed to human activities driving dispersal of dog-associated lineages[147]. A high velocity of dog-meditated rabies (18.1 km/yr), was estimated in Iran, using a novel analytical framework, revealing spread linked to accessible areas associated with high human density[45]. This estimate is similar to the average dispersal rate in Northern Africa (19.5 km/year) where, again, landscape accessibility was an important driving factor[15,45].

The time of introduction and history of rabies spread in various areas was investigated. A publication used historical records and phylogenetic analysis to show that rabies was only present in bats and skunks in the Western Hemisphere, with canine rabies rare or absent among dogs of Native Americans, before the arrival of new dog breeds imported during European colonisation[56]. A more comprehensive recent study combined partial and whole genome sequences to reconstruct movement more precisely, revealing how colonial empires influenced the global spread of rabies viruses[148].

Human-mediated long-distance movement was identified through phylogeographic analysis as the source of incursions into previously rabies-free areas, posing a challenge to maintaining rabies freedom. Examples identified introductions to historically rabies-free areas such as island provinces in Indonesia[138] and the Philippines[78], and to areas where rabies had been eliminated such as Pemba, (an island off mainland Tanzania[149]), Gauteng Province in South Africa, and N'Djamena, Chad[40].

Geographical features were discussed in fifteen papers, with rivers, lakes and mountain ranges shown to be natural barriers[15,150,151]. For example, sequences within AF1b subclades (AF1b-1 and AF1b-11) were separated by the Zambezi river and Kariba lake in Zambia and Zimbabwe respectively[150]. Transboundary spread between Lesotho and KwaZulu-Natal in South Africa was

limited by the Drakensberg and Maloti mountain ranges, which constrain movement of people and animals[151]. In Asia, three lineages from SEA5 found in ferret badgers were segregated by mountain ranges and rivers[111,119]. Transmission corridors that facilitated dissemination included: (1) transportation networks, (2) the presence and size of dog populations (tied to human populations) and (3) anthropogenic factors (trade, agriculture and urbanisation). For example, in Southeast Brazil, urbanisation was reported to play a central role; dog population size was correlated with human populations, meaning higher density regions had more dogs and therefore more RABV diversity[152]. Road networks were often shown to be associated with increased movement, consistent with human-mediated transport of incubating animals. RABV detected from different cities in Mozambique were closely related, despite long distances between them[100]. Sequences from the AF2 clade were proposed to result from an introduction to Bangui, the capital of the Central African Republic, as the closest related sequences were from neighbouring Chad[41]. In Asia, transport routes were correlated with the RABV distribution in Thailand[153], and China[154]. At more local scales in Tanzania phylogeographic analysis revealed that presence of dogs, rather than density, predicted spread[155].

Rabies control

All studies provided recommendations for rabies control and prevention, but most were generic and not specifically inferred from sequences. These studies recommended mass vaccination of dogs, as well as oral vaccination in specific wildlife populations where variants had emerged[156,157]. Other recommendations included dog population management[76,113,158,159], monitoring the health of animals for trade and consumption[75,80,97,129], and raising awareness, particularly in communities identified as "high-risk"[155,158,160,161]. Only 33 publications provided targeted recommendations grounded in genetic evidence. Some reported spillover events, emphasising the necessity for enhanced surveillance (n=12) for specific wildlife populations[59,72,93,95]. Outbreak investigations pinpointed sources of incursions, and recommended monitoring for animal transport and at borders[78,82,138,162] plus surveillance and control measures in rabies-free areas or areas with low-incidence, where introductions pose risks[89,149]. Likewise, identification of novel variants and genetic diversity prompted suggestions for oral vaccines and baits tailored to hosts, e.g. for ferret badgers[118,143]. There were no instances of vaccine-derived cases/outbreaks in the review, but genomic surveillance would be a crucial tool to identify and monitor such occurrences.

Widespread coexistence of diverse lineages and insights into their evolutionary history, transmission dynamics and dispersal rates from more complex phylogeographic analysis, highlighted the urgency of addressing transboundary transmission, which requires coordinated

effort[65,80,140]. Vaccination campaigns focusing solely on urban localised dog populations have demonstrated short-lived success due to rabies circulation across land borders. Examples include Chad and Central African Republic[40,41], India and Bhutan[58], and Peru and Brazil bordering Bolivia[163]. The same was true between islands, for example Pemba, off Tanzania[155], and within and between archipelagic countries in Southeast Asia[78,108,138] and also was identified at the borders of states in India[164]. Therefore, recommendations included scaling up dog vaccination beyond urban centres to encompass surrounding rural areas, along with coordinating transboundary dog vaccination to minimise spread into cities or between neighbouring countries or administrative units such as states or provinces[40,41].

DISCUSSION

In this review, we focused on how genetic data informs understanding of rabies dynamics and its control. Findings from 220 studies demonstrate sequencing as a potentially powerful tool for contributing to 'Zero by 30'. However, information from sequences is often not fully or consistently synthesised into specific, actionable recommendations. When used effectively, sequencing has been instrumental in tracing incursions into rabies-free areas and highlighting extensive transboundary transmission that necessitates coordination of control nationally and regionally[78,138,149]. Although RABV diversity differs across these regions with endemic dog-mediated rabies, spillovers and transboundary movement was repeatedly reported, emphasising the importance of coordinated transboundary vaccination efforts and surveillance. As countries approach the "*endgame*", *i.e.* the final stages of an elimination programme where disease is still circulating but at much reduced levels, genetic data is expected to become increasingly useful, providing greater insights for monitoring emerging issues such as spillover and adaptation to alternative hosts and potential re-emergence in dogs.

A major challenge is how classification of phylogenetic diversity and associated nomenclature, beyond the clade level, is not standardised and how varied terminologies (subtypes, subclades, subclasses, clusters etc) are used. In most publications from Africa and Asia, groupings were designated numbers or letters based on subjectively defined clusters in phylogenies, while Latin American studies often employed antigenic variant classification rather than evolutionary (phylogenetic) relationships. Inconsistent terminology hampered a clear understanding of circulating lineages and their geographic distribution, and hindered their use as reference points for further research[42]. Many studies used nomenclature only meaningful within the context of that particular study and classifications often differed across related studies (Supplementary Table 1); employing RABV-GLUE[42] to classify minor clades revealed these inconsistencies (Figure 4). The significance of discerning lineages lies in implications for control, for example,

differentiating incursions from undetected local transmission and identifying the scale of circulation. Variations in the portion of the genome sequenced (gene-specific) and sequence length contributed to inconsistencies. Most publications used partial genome sequencing, often targeting the N gene, a relatively conserved region used as a diagnostic marker. But, this limited sampling missed important variation, which may define rare and divergent lineages or improve resolution across narrower spatio-temporal windows. Just 20% of publications used WGS, which can provide deeper understanding.

We found an urgent need to increase in-country sequencing capacity in endemic countries, with relatively few generating sequences in-country. Regionally, Africa lagged behind Latin America and Asia in terms of capacity, but had a higher output than Latin America, likely because few countries in the region remain endemic for dog-mediated rabies. Most sequencing was driven by research, with routine sequencing not yet part of surveillance in endemic regions. Consequently, there is a shortage of sequences, with largest contributions from China and Brazil (upper-middle-income countries with existing networks and resources), whereas several countries had limited representation; some with just one publication. The scarcity of sequences, which poses a challenge to characterising RABV diversity and understanding transmission, could be due to different cultural, intrinsic and socio-economic factors. In the aftermath of the COVID-19 pandemic, sequencing capabilities have expanded, becoming more affordable and accessible. This increased capacity has practical applications for responding to rabies outbreaks[149].

Although dogs were identified as the primary host responsible for most transmission in regions with endemic dog-mediated rabies which were the focus of this study, RABVs broad host range means it is capable of transmission among multiple species. This versatility creates potential for new reservoirs, raising concerns about the effectiveness of control measures, and about these reservoirs being a source of re-emergence[160]. In areas where dog-mediated rabies has largely been eliminated, there were several examples of transmission cycles in wildlife, some of which resulted from spillover from dogs. In Latin America, where canine rabies incidence has reduced dramatically from coordinated regional control, intriguing reservoir dynamics have emerged. For example, three major enzootic cycles, distinct from dog rabies foci in Mexico (skunks, coyotes and gray foxes) highlight a complex maintenance dynamic[165]. Similarly, transmission cycles have established in ferret badgers independently in both Taiwan and China[118,119,166]. These findings underscore the complex reservoir dynamics of RABV and importance of understanding host associations and cross-species transmission. As dog-mediated rabies declines, this understanding will be necessary to enable targeted control, either directing efforts at blocking transmission and spread between source and target populations or controlling infection within new reservoirs. The

risk of rabies spillover from wildlife foci to dogs is a serious challenge for 'Zero by 30'. When such reservoirs exist, tailored surveillance and control measures are crucial to mitigate the threat of re-emergence in dog populations as a result of spillover. This may eventually require countries to undertake control measures in wildlife reservoirs, but more imminently underscores the need to eliminate rabies from dog populations before wildlife foci can establish.

Underreporting and misdiagnosis of human rabies remains a significant issue and can create a false impression of low burden in rabies endemic settings across Africa and Asia. While enhancing diagnostic capacity and overall surveillance is crucial to address underreporting, we also emphasise the pivotal role that genetic data can play in strengthening human rabies surveillance. Rabies-free countries experience imported human cases from exposures in endemic countries[167–169]. Oftentimes, these imported cases were confirmed through epidemiological investigation, but genetic data identified the sources of infections, as well as ambiguous cases, without a clear route of exposure (e.g. no bite history) or origin (e.g. the migrant from Mexico and the veterinarian in Brazil), or with unusually long incubation periods[133,135–137]. These human cases identified from countries that are free from dog-mediated rabies highlight how these technologies could be applied to strengthen human rabies diagnosis and source attribution within endemic countries.

Unlike infections in humans, which are effectively dead-end hosts, movement of infected animals is the predominant factor contributing to RABV establishment in new geographic settings. Publications highlighted both local host movement and long-distance human-mediated movements, however, evidence on transmission links and directionality were sparse, with only a few studies (25%) employing phylogeographic analyses. Most used genetic relatedness of viruses from different locations, based on interpreting phylogenies, without quantitative inference. When carried out, phylogeographic analyses illuminated environmental features as both natural barriers and drivers of spread, with human-related factors driving dispersal towards more populated and accessible areas. These analyses have potential to guide spatial targeting of vaccination, and enhancement of surveillance in at-risk areas[45,89,170]. Frequent reintroductions highlighted epidemiological connectivity of landscapes over which vaccination needs scaling and coordination across political/ administrative boundaries,[40] with more recent studies showing how sequencing can be used to monitor the impacts of and threats to dog vaccination programmes[40,98,149].

Broader context

Our review has relevance to the broader application of genetic surveillance to pathogens. The recent increase in third-generation sequencing of RABV has potential to further expand given

the focus on sequencing capacity for pandemic response. Deployable sequencing has become a key component of outbreak response, with portable lab equipment and sequencing platforms facilitating on-site, real-time, genomic surveillance[16,23,26]. Feasibility and utility of deployable sequencing for rabies surveillance has been demonstrated[29,98,149], with use of Nanopore's MinIon reducing costs and turnaround times[17,29], and comprehensive protocols, bioinformatic pipelines and open-source user-friendly software and classification tools becoming more available[171–175]. Sequencing for routine surveillance of endemic zoonoses could build and sustain pandemic preparedness at the human-animal-environment interface. While COVID-19 accelerated application of genomic surveillance, it also highlighted stark global disparities in access to sequencing, and bioinformatic expertise remains a bottleneck[176,177] Since the pandemic investment in LMICs has begun but much more is required[178].

RABV also serves as a model system to understand and manage cross-species transmission and spillover. Instances highlighted in this review, reflect a broader ecological pattern with significant public health and ecological implications. Spillovers can be precursors to larger outbreaks, as evidenced by recent epidemics of Influenza, Ebola, Zika, and COVID-19, while swift sequencing and analysis can inform public health responses and containment[16–18,23,26].

Limitations of the study

While we endeavoured to comprehensively review global regions with endemic canine rabies, it is important to acknowledge the limitations inherent in our study. Genetic studies on rabies demonstrating progress in its elimination, conducted in countries or regions such as Canada, the USA, and Eastern Europe, where dog rabies has been eliminated for some time, were not included. This approach may have limited our ability to discover additional interventions and significant insights from the perspective of these countries. Our review, while focusing on publications from endemic regions may have failed to identify publications documenting importations in rabies-free countries. We supplemented our searches by manually adding relevant instances (~10% of papers), but some studies, particularly those published in non-English journals, may have been overlooked. Despite substantial manual curation efforts in RABV-GLUE to enhance GenBank metadata[179], inconsistencies and gaps persist, potentially hindering data mining efficiency for sequences not associated with a publication. We also only retrospectively and manually identified some studies that did not report the use of sequencing in their abstract. Not all papers transparently report methods, in particular many did not report locations where sequencing was undertaken which we assumed was done in-country. We therefore likely overestimated sequencing capacity for some countries, though we expect our conclusions are robust. We included only studies published after the year 2000, which limited the scope of the study, as it did not capture some of the earliest genomic studies conducted on

RABV. However, this also meant most of the methods reported were more comparable and aligned with the 1st, 2nd and 3rd generation sequencing platforms defined in Box 1.

Conclusions and future recommendations

The promise of genetic data for informing rabies control is evident, but its full potential remains untapped as most publications advocate generic measures that lack specificity derived from sequencing. More demanding phylodynamic analysis, integrating geographical, epidemiological, and genetic data to yield more detailed and quantitative understanding, requires greater expertise and computational resources that have not been accessible in LMICs. Future research would benefit from more WGS as well as leveraging the power of existing data through analyses that integrate partial and WGS[148]. Applied insights to be gained by enhancing rabies surveillance with sequencing, lie in the knowledge of what is circulating and how it is spreading while we gear towards elimination. A standardised nomenclature system for categorising RABV diversity, would facilitate clear communication and collaboration among researchers, healthcare professionals and policymakers[175]. We recommend efforts to develop a robust taxonomic classification system under the International Committee on Taxonomy of Viruses (ICTV) that is capable of integrating all existing and newly identified RABV sequences. Scaling up sequencing in endemic countries, with laboratory networking and a more unified terminology for exchange of information and updates on new variants and lineages could enhance risk assessment and control strategies. Genetic results underscore the need for international and regional coordination in controlling transboundary spread, to accelerate progress and maintain gains. Expanding sequencing initiatives and fostering collaborative efforts will support the 'Zero by 30' goal, and serve as a prime example of a genomics-informed One Health approach, building capacity for the future[180].

DECLARATIONS

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None

Authors' contributions

Design: Kirstyn Brunker, S.M. Thumbi, Katie Hampson, Julius O. Oyugi

Literature search: Gurdeep Jaswant, Criselda T. Bautista

Data analysis: Gurdeep Jaswant, Criselda T. Bautista, Katie Hampson, Kirstyn Brunker, Brian Ogoti, Joel Changalucha, Kathryn Campbell, Mumbua Mutunga

Manuscript writing: Gurdeep Jaswant, Criselda T. Bautista

Manuscript editing: Katie Hampson, Kirstyn Brunker

Data source and availability

Data and code to reproduce the analyses and figures are available from our public repository https://github.com/RAGE-toolkit/RABV_geneticSurv_review

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Conflicts of interest

There are no conflicts of interest.

Patient consent

Not applicable

Ethics approval

Not applicable

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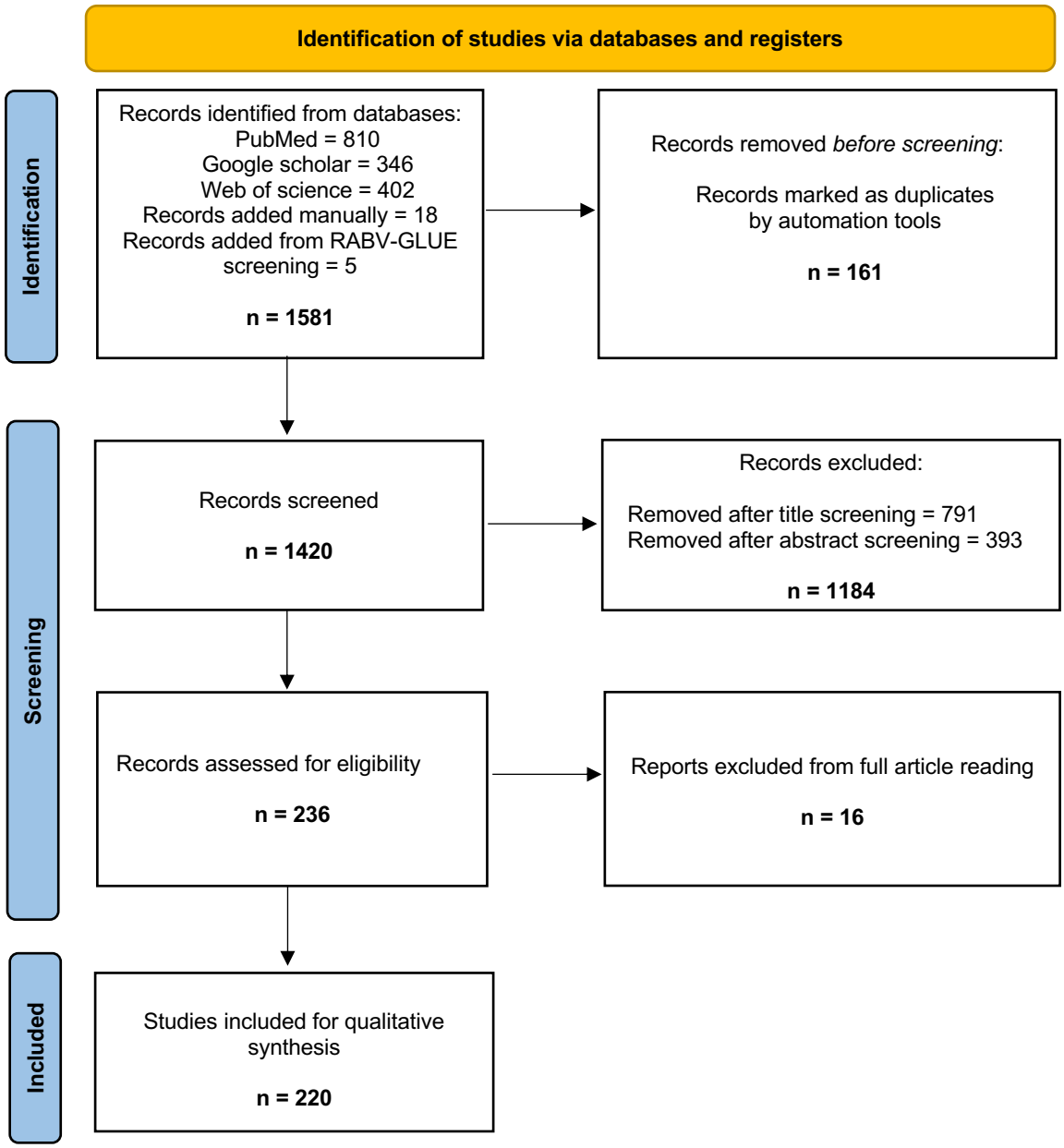
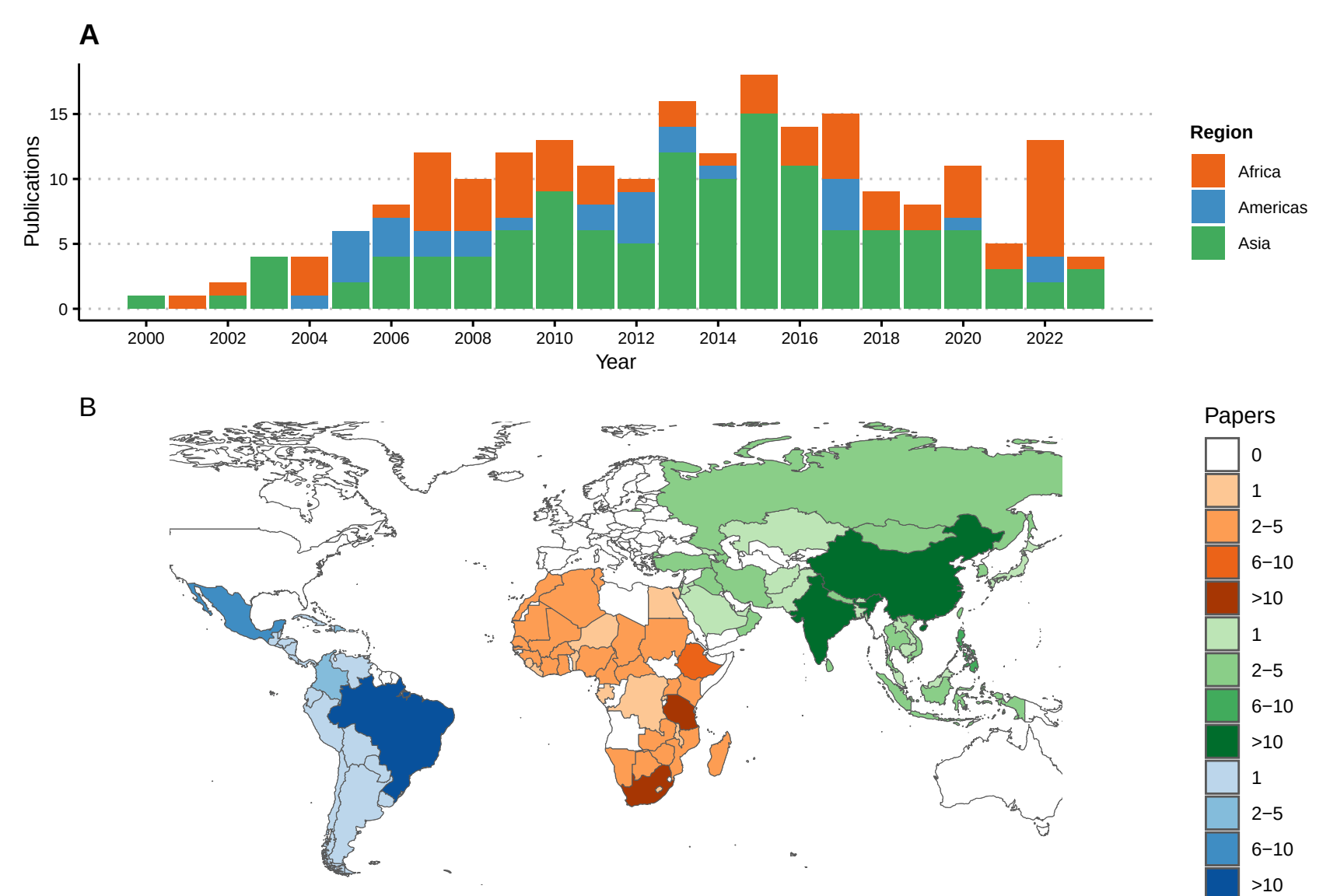
PRISMA 2020 flow diagram for new systematic reviews which included searches of databases and registers only

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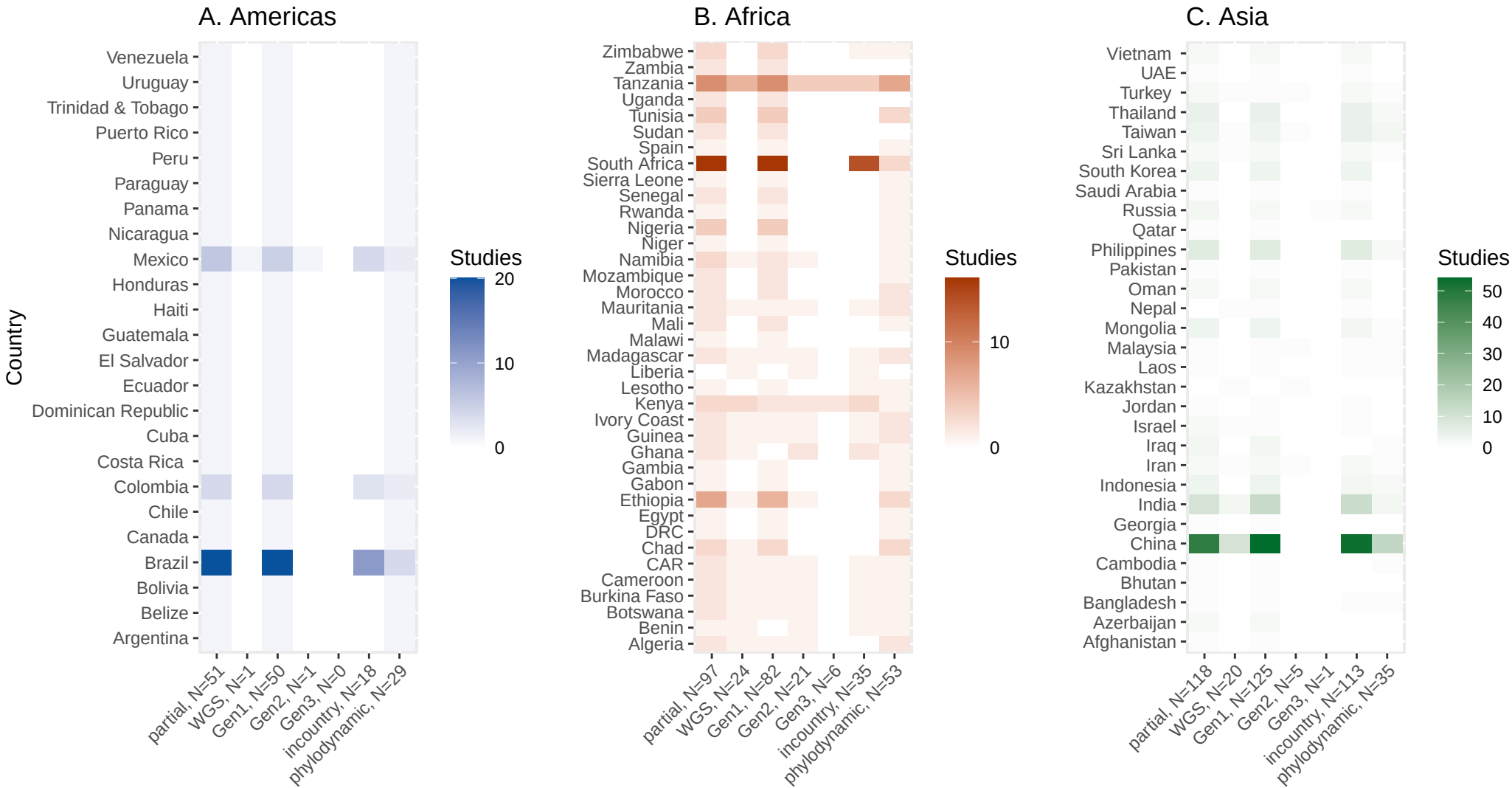
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Dominican Republic
Cuba
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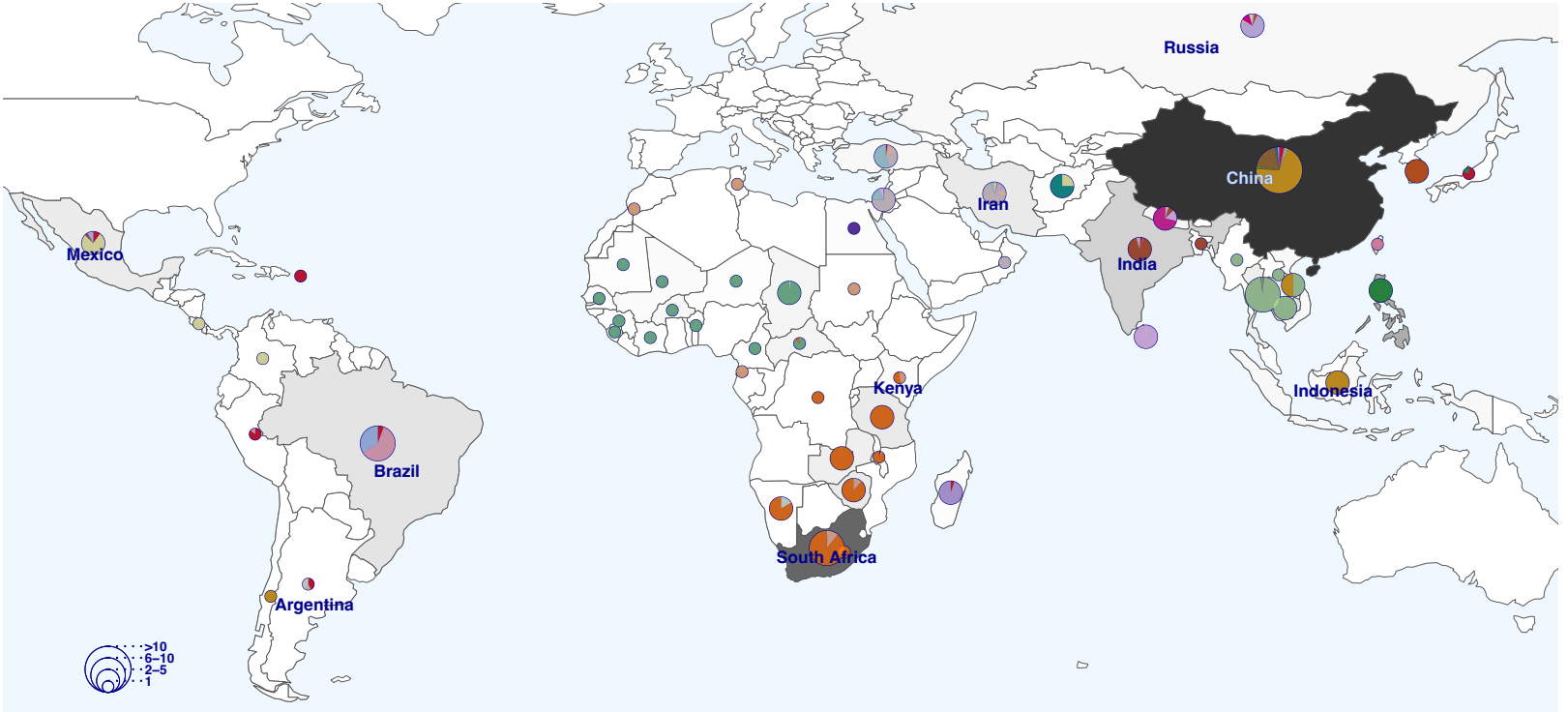


Number of sequences

1250
1000
750
500
250

Phylogenetic clade (Major.Minor)

Africa.2	Arctic.AL1b	Asian.SEA2a	Cosmopolitan.NA	Cosmopolitan.AM2a	Cosmopolitan.CA1	Cosmopolitan.ME2
Africa.3	Arctic.AL2	Asian.SEA2b	Cosmopolitan.AF1a	Cosmopolitan.AM2b	Cosmopolitan.CA2	Cosmopolitan.Vac
Arctic.NA	Arctic.AL3	Asian.SEA3	Cosmopolitan.AF1b	Cosmopolitan.AM3a	Cosmopolitan.CA3	Cosmopolitan.Vac2
Arctic.A	Asian.SEA1a	Asian.SEA4	Cosmopolitan.AF1c	Cosmopolitan.AM3b	Cosmopolitan.ME1a	Indian.Sub
Arctic.AL1a	Asian.SEA1b	Asian.SEA5	Cosmopolitan.AF4	Cosmopolitan.AM4	Cosmopolitan.ME1b	Unclassified

Response letter

Reviewer #1 Comments and reply:

This review conducted a comprehensive search of bibliographic databases for primary research on rabies published since 2000, and extracted and synthesized surveillance data to provide recommendations for further research and application to support rabies control. It has strong research significance. These are the following points that need to be added or revised.

1.Authors said that viral sequencing can support the 'Zero by 30' goal. I think that " viral sequencing can support the trend understanding and the risk assessment of the epidemic at the global level, and the formulation and implementation of prevention and control measures for the 'Zero by 30' goal" is more accurate. The corresponding part of the article should be revised.

Thank you for the comment, we have revised the statement accordingly: "This systematic review investigates how viral sequencing can contribute to achieving the 'Zero by 30' goal by improving understanding of viral circulation and the impact of rabies control measures." (lines 30-32)

2.The limitations of the work should be added in discussion.

We have now added a sub-section "Limitations of the study" under the discussion as per the suggestion and have detailed limitations in the section. (lines 572-587)

3.The important results for different regions or countries should be compared.

Thank you for this comment. We did not draw out the full comparisons, which we have now tried to improve, particularly the results section on circulating RABV (lines 205-206, lines 230-233). Comparisons within regions are reported in these paragraphs too (Africa, lines 212-219, Asia lines 219-225, Latin America lines 226-228) and comparisons of their nomenclature are on lines 248-256. We also compare differences in dispersal rate reported from countries globally (lines 384-390) and general features that appeared to support rabies spread across regions (lines 404-422).

In the discussion, we also compare the naming systems used among the endemic regions "In most publications from Africa and Asia, groupings were designated numbers or letters based on subjectively defined clusters in phylogenies, while Latin American studies often employed antigenic variant classification rather than evolutionary (phylogenetic) relationships." (*lines 471-474). Also the sequencing capacity within the three regions was compared in the discussion "Regionally, Africa lagged behind Latin America and Asia in terms of capacity, but had a higher output than Latin America, likely because few countries in the region remain endemic for dog-mediated rabies." (lines 489-491). Reservoir dynamics were also compared in the discussion highlighting examples of independent establishment of wildlife foci that resulted from spillover of dog-mediated rabies. In light of these points of comparison, we have added a further sentence to the first paragraph of the discussion reporting similarities across regions. "Although RABV diversity differs across these regions with endemic dog-mediated rabies, spillovers and transboundary movement was repeatedly reported, emphasising the importance of coordinated transboundary vaccination efforts and surveillance.".(lines 461-463)*

Reviewer #2 Comments and reply:

Dear Authors,

I would like to draw attention to some discrepancies in the manuscript that it would be desirable to explain or correct.

1. According to the eligibility criteria, "studies had to address either dog, human or terrestrial wildlife rabies (i.e. not bat rabies)". The authors declare that "…goal was to examine the extent of the application of genetic approaches to RABV surveillance globally…" But judging by the selected publications and analysis results, the main focus is on Asia and Africa, where dog rabies is widespread.

Our primary focus is on regions with endemic dog-mediated rabies. While Latin America has largely eliminated dog-mediated rabies, there are still pockets of endemicity (e.g. Bolivia, parts of Peru). Many publications from Latin America emphasise wildlife-associated variants, but we include studies that identify transmission in dogs and dog-associated or dog-derived variants.

We have now revised this part of the introduction to make this clearer: "In this systematic review, our goal was to examine the extent of the application of genetic approaches to RABV surveillance in regions with endemic dog-mediated rabies (Africa, Asia, and Latin America) and how, going forward, these approaches can contribute to the 'Zero by 30' goal." (lines 113-116). We also now better highlight this focus in the methods section of the abstract (lines 33-36) Within the methods, we added a sentence into the screening part about how we excluded studies that were not covering rabies in Asia, Africa and Latin America. (lines 141-143).

2. Data for North America and Europe are sometimes given in the text, but Figures 2-4 sometimes present misleading information. For example, from Figure 2 it seems that genetic research has not been carried out in the USA, Canada, Poland, Finland and a number of other European countries after 2000. As is known, dogs are not a reservoir of the rabies virus in these countries, but in rare cases, they can serve as a vector for humans.

North America and Europe were mentioned in the manuscript as non-endemic regions that reported human rabies cases where the source of exposure was thought to be from endemic countries.

In the map, we included the identified country of origin (i.e. exposure location) of these cases in the publications and not the country where the human case was detected (with humans considered dead-end hosts).

In the manuscript, under the human rabies section, we explain: "Eight were traced to immigrants with exposure histories in endemic countries where PEP was not received before travel to the country of diagnosis. Analysis of the patient isolates revealed similarity with cases in postulated countries of origin, including a case in the UK imported from Nigeria and a case in France imported from Mali, two unrelated cases in Qatar both imported from Nepal; and cases imported from the Philippines to the UK, Japan and Finland, as well one that likely originated from insectivorous bats in a Mexican immigrant bitten by a fox, who died in California. Similarly a sequence from a patient in France, with recent travel history to Mali but no known exposure, belonged to the AF2 subclade that circulates in West Africa". (lines 322-331). We are sorry for the confusion and hope our reasoning is clearer now and is also highlighted in the discussion (lines 529-535).

3. Several places in the text mention that spillover from dogs into other carnivores often occurs. But currently, in many parts of the world, the situation is the opposite: the main reservoir of various variants of the rabies virus are wild animals, and interspecies transmission from them to dogs is important. And this has a direct bearing on the project 'Zero by 30'.

This is a very important point from the reviewer. Spillover of rabies from wildlife into dogs does represent a challenge for 'Zero by 30'. When such reservoirs exist, surveillance and control measures may need tailoring to mitigate the threat of re-emergence in dog populations as a result of spillover. In this review, we more commonly find examples in endemic areas of dog rabies spilling over into wildlife hence this was more our focus. If this spillover does establish in wildlife populations, these new reservoirs could similarly threaten 'Zero by 30'. Recognizing these risks, and the importance of genomic surveillance being able to distinguish these scenarios, we now elaborate on this in our discussion (lines 507-522).

4. Specific comments are given below line by line. In addition, sections of the text where, in my opinion, editing is required, are highlighted in yellow in the manuscript. Including notes made in the list of references.

We have addressed the editing concerns in the list of references, making sure everything is in the same style of referencing.

5. Probably, it would be better to place Box 1 in the supplementary materials, and not in the body of the article.

For now, we have decided to keep Box 1 within the main manuscript so as to easily refer to key definitions when necessary, but are happy to move to the supplementary materials if advised by the editor.

6. A table with the original local names of the subclades and lineages of the rabies virus in comparison with modern names (Figure 4) would be useful to add to Box 1, especially for a cosmopolitan clade.

We have now included a statement within the Definition Box referring to a table where you can see the different local names of subclades and lineages (publication designation) in comparison with the name we applied using RABV-GLUE designation (lines 116-117).

7. The list of references includes 178 publications, but at the beginning of the manuscript, it is stated that 220 was selected for analysis. Did I understand correctly that the list was formed selectively?

The publications were identified through the systematic screening and review process, which we elaborate on in the manuscript methods and Figure 1. In total, 220 publications were included in this process, but only a subset are directly cited in the manuscript, along with other relevant wider literature. The full list of publications included in the systematic review is provided in Supplementary Table 4 along with the corresponding data extracted from each. We summarise this information in the main figures and text.

8. Line 49 "nomenclature for rabies viruses" / nomenclature of intraspecific variants (genetic lines) of the rabies virus

Thank you for the comment, we have revised the statement: "There is an urgent need for standardised classification methods and phylogeny-based nomenclature for rabies viruses,

and for improved sequencing capacity in rabies endemic regions, including proficiency in bioinformatics and phylogenetics." (lines 48-51)

9. Lines 94-95 "Its susceptibility to drugs" / In an article about rabies, this could not be written about, since there is no specific medicine.

We were referring more generally to sequencing as a tool for pathogen surveillance but to avoid confusion we have revised accordingly: "Increasingly, surveillance involves genetic data, for pathogen diagnosis and risk assessment, as well as to identify the source of outbreaks and to characterise pathogen spread." (lines 94-96)

10. Lines 97-98 …" for pathogens such as Influenza16,17, Ebola18, Zika19, Yellow fever20,21, Mpox22–25 and SARS-CoV-2" / We are talking about pathogens, but the names of diseases rather than viruses are listed below (with the exception of SARS-CoV-2)

Thank you for flagging this, we have added the virus name now to be clearer that we are talking about pathogens: "Influenza virus, Ebola virus, Zika virus, Yellow fever virus, Mpox virus and SARS-CoV" . (lines 97-99)

11. Line 131 "sequenc" / sequence???

The asterisk after "sequenc" denotes the search is for text with variable endings after "sequenc" e.g. sequence, sequences, sequencing*

12. Title Figure 4. "The spatial distribution of canine rabies virus (RABV) clades"/ What does canine rabies virus? Belonging to the domestic dog, the canid family, or as a clade distinct from bat viruses?

Sorry we were not clear here. We have revised the title to say "dog-associated rabies virus (RABV) clades". We do not include bat viruses". (line 258)

13. Line 216 "four clades: Cosmopolitan, Arctic-like, Indian Subcontinent and Asian" / In the following, in various places in the text, information is also given about clade Arctic, which is sometimes considered as a clade of a higher order in relation to Arctic-like. In other cases they are described as two separate clades. From an epidemiological point of view, the distribution areas of these clades are isolated from each other. Clarifications or corrections would be helpful. (see also lines 242, Fig.4)

Thanks for your comment. The Arctic clade, its subclades (including Arctic-like viruses) and and lineages are disparately distributed geographically (across North America, Northern Asia and throughout much of the Asian continent) despite sharing a common ancestor. The terminology present in the literature is sometimes inconsistent with references to both Arctic and Arctic-like viruses in different publications, while the clade origin is also sometimes misinterpreted due to the geographical name associations.

To avoid ambiguity we used RABV-GLUE for comparing clades, which uses an algorithm that requires sufficient sequences to allow genetic differentiation of clades. However if insufficient sequences are publicly available, RABV-GLUE will not be able to classify the virus to high resolution so will default to the parent (well-defined) clade (Arctic) even if a higher resolution (Arctic-like clade) is referred to in the original publication. This is why we note that inconsistencies with the names used in publications may be seen and why we provide a key (see Box 1). The Arctic and Arctic-like clades is a good example of where a consistent nomenclature is required, that is robust to accumulating sequence data, and this is a major conclusion of our study. We hope this explanation helps with clarification, which we have also tried to make clearer in the main text.

14. Line 265 “Most livestock outbreaks were attributed to dog-mediated transmission” / This is doubtful on a global scale. In the USA, Europe, and northern Asia, livestock becomes infected mainly from foxes and other wild mesocarnivores.

This is a good point which we did not communicate clearly. We have revised the statement to explain that we are referring to livestock outbreaks in regions with endemic dog-mediated rabies: “In regions with endemic dog-mediated rabies most livestock outbreaks were attributed to spillover from dogs, except for outbreaks in Latin America due to vampire bat rabies. However, spillover from wildlife to both dogs and to livestock was also observed, as seen in a livestock outbreak linked to foxes in northwest China.” (lines 275-279)

15. Line 269 “Domestic dogs were predominantly identified as the reservoir of RABVs”. This is also not correct for the whole world.

We have corrected this and added “within the defined scope of the study” to be more specific. (lines 281-282)

16. Line 290 “The only exception was the novel Ikoma lyssavirus (IKOV), isolated from an African civet killed after biting a child in Serengeti National Park124. West Caucasian Bat…” These are other species of the Lyssavirus genus. As stated at the beginning of the manuscript, bat lyssaviruses were not analyzed.

The reviewer is correct that this example identifies a lyssavirus. The reason the study was included, is that the publication indicated this was an unusual case expected to be the result of dog-mediated spillover, however sequencing refuted this (and led to the recognition of ikomavirus). We have clarified this now. (lines 304-309)

17. Lines 300-301 “Two were bat origin” /Again about bats…

Bat rabies studies were excluded as part of the selection process of publications for this study. However in a small number of cases the sequence data from studies in regions with endemic dog-mediated rabies did point to bats as the origin of rabies cases rather than of dog-mediated rabies. This was a difficulty for the sequenced human cases, which also often involved complicated immigration histories. We opted to include these studies rather than include them because the case data reported would raise suspicion of dog-mediated rabies. Moreover, these cases underscore the value of genetic data in determining the origin of human cases, which is one of the important conclusions of our study. We hope that our clarification in the text helps.

18. Line 327 “Virus movement” / rabies virus movement

Thanks, we have revised as suggested to “Rabies virus movement”

19. Line 353. “extensive land borders like Azerbaijan”90 / In link 90 there is an article about the situation in Bangladesh. Besides, it would be better not to put the length of the land border between China and Azerbaijan on the same level. There are many more suitable examples.

We have revised this statement, adding other examples: “RABVs circulating in neighbouring countries were often closely related, particularly across shared land borders, like Azerbaijan with Georgia, Bangladesh with India and Tibet with Nepal.” (lines 373-375)

20. Line 524 “Covid “/ COVID-19

Thanks, we have corrected this. (line 562)

Reviewer #3 Comments and reply:

I have comments:

1. Line 126: Why were only studies included published after the year 2000? This seems arbitrary. The period of included studies should be based on the availability of sequencing methods.

We chose the year 2000 expecting that sequencing became more mainstream this century (particularly in rabies-endemic countries) and because we needed to choose a starting point so that the number of included papers was feasible. However, we agree that this was an arbitrary cut off, and now report this as a limitation of the study: "We included only studies published after the year 2000, which limited the scope of the study, as it did not capture some of the earliest genomic studies conducted on RABV. However, it also meant most of the methods reported were more comparable and fitted within the 1st, 2nd and 3rd generation sequencing platforms defined in Box 1." (lines 583-587)

2. Line 175: The number of total included countries in the 220 studies was 94. Here you mention 92. Does this mean that 2 countries were not endemic and hence the sequences reported were travel associated cases as mentioned in caption of figure 2? If yes, specify it in the text, too, to avoid confusion.

Thanks for picking this up. We have corrected this to 94 as this was the original number of countries where sequences were generated from in the studies. The five studies reporting travel-associated human cases were counted according to the country of origin (detailed in our response to reviewer 2). (line 178-180)

3. Line 201: You included 220 articles in the review. Assuming that 1 article = 1 study, the 92 mentioned here are not "most" in my opinion. Please clarify or change to many instead of most.

We have corrected the statement to "with many studies (n=92)" (line 209)

4. Line 293: Does N=18 refer to the studies or to the exposure cases in the studies. How many human exposure cases with sequences were published in total across all the 220 articles? Is it 22 (18+4)?

Thanks to your inquiry, we have updated this section accordingly. To summarise, we now have a total of 24 publications that specifically investigate human rabies cases. We have added two more studies to this count. These were previously referenced in other sections (therefore the overall number of studies, 220, remains unchanged) but were mistakenly not included in the count for human papers. Collectively, these publications included sequences from 219 human samples. (lines 312-320), the majority of which came from a large study conducted in South Africa (127 human sequences) and Sri Lanka (36 human sequences). Most other papers focused on the investigation of only 1 or 2 human cases.

5. Line 305: This sentence is confusing. If the dog died of unknown causes how could phylogenetic similarity be established with the dog case? If the dog had died of rabies and a sample was taken it cannot be an unknown cause of death.

Apologies, we wrote this in a confusing way and have now revised the statement to be clearer "The second case, a 6-year-old from KwaZulu-Natal, had no bite history, however his neighbour's dog died of unknown causes around the same time and phylogenetic similarity of sequences from the neighbours' dog and the child suggested the child was exposed from the neighbour's dog". (lines 343-346)

6. Line 434: Explain what you mean with “endgame”. I am familiar with the term, but not all readers might be.

We have added this into the document to explain more a bit about “endgame”: As countries approach the “endgame”, i.e. the final stages of an elimination programme where disease is still circulating but at much reduced levels, genetic data is expected to become increasingly useful, providing greater insights for monitoring emerging issues such as spillover and adaptation to alternative hosts and re-emergence in dogs. (lines 464-467)

7. Formatting issues: Format text either left align or justified, currently it is a mix of both.

Thanks for this comment, we have addressed this and used justified format for the whole document.

8. Supplementary files:

The text font is not the same throughout in the supplement file 1. Please harmonize.

Thanks. We have edited Supplementary file 1 using one font throughout.

9. Table captions need to be on top and not on the bottom of tables. All supplementary tables are very cumbersome to read and need formatting! Supplementary table 3 does not have a caption.

We have revised the supplementary files, including formatting and placement of table titles. We will be sending a new folder of all the revised supplementary files listed below:

Supplementary File 1. Viral Sequencing to Inform the Global Elimination of Dog-mediated rabies - Protocol

Supplementary Table 1. A table of publication-specific clade designations and corresponding RABV-GLUE designations

Supplementary Table 2. Search terms used to select publications.

Supplementary Table 3. Example of the MeSH terms used to select publications in the PubMed database search.

Supplementary Table 4. Selected publications and extracted data

Supplementary Figure 1. Summary of RABV sequencing by country, including the sequencing platform, type of sequence generated, location of sequencing and analyses undertaken

Reviewer #4 Comments and reply:

1. The manuscript is scientifically sound. The few hitches are pointed out in the attached manuscript. Responding to the queries and comments should improve the manuscript.
Thanks for this, we have addressed all your edit suggestions and highlighted it in yellow within the manuscript.

2. This figure is too small to aid good readability. It can be accompanied by supplementary materials (bigger, more legible versions of the same figure) arranged as A, B, and C.
We are liaising with the editor to request the figure can be fullpage for publication. For now we have created a supplementary file showing an enlarged version of the figure arranged per study regions, with labels A, B and C and state that an enlarged figure is available as Supplementary Figure 1".

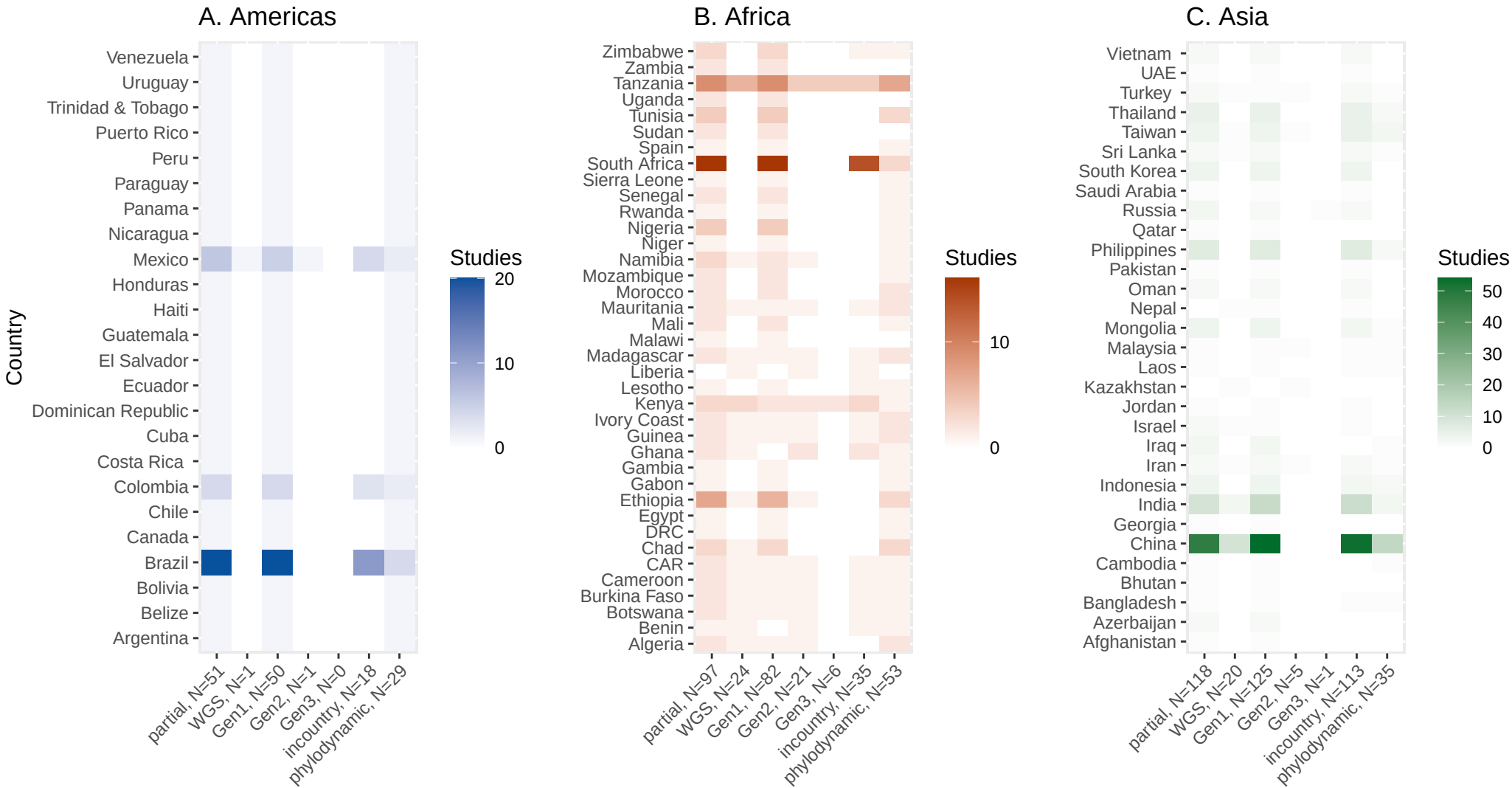
3. It should also be mentioned that 'the basis/origin of these dearth in genetic sequence should be investigated'. Although, we may think that it is simply due to lack of resources, there may be other insidious/in-apparent reasons, cultural, guttural, socio-economics, etc.
Thank you for the comment, we have revised this statement: "The scarcity of sequences which poses a challenge to characterising diversity and understanding transmission could be due to different cultural, intrinsic and socio-economic factors". (lines 495-497)

4. Line 456: "Inconsistent terminology hampered a clear understanding of circulating lineages and their geographic distribution….." With this major gap, I will expect the authors to make categorical statement on this, asking for the global Rabies research team or ICTV to standardize the nomenclature of rabies, and possibly reclassify all the available isolates to date using this standards.
Thank you for highlighting this. We have now stated in the conclusion and recommendation statement: "A standardised nomenclature system for categorising RABV diversity, would facilitate clear communication and collaboration among researchers, healthcare professionals and policymakers175. We recommend efforts to develop a robust taxonomic classification system under the International Committee on Taxonomy of Viruses (ICTV) that is capable of integrating all existing and newly identified RABV sequences." (lines 598-602)

5. Line 494: "Underreporting and misdiagnosis of human rabies remains a significant issue and can create a false impression of low burden….." This appears to be the situation in several areas of Africa and Asia, where rabies is thought to be endemic. The authors must be bold to mention this.
Thank you for this guidance, we totally agree. We've revised the statement: "Underreporting and misdiagnosis of human rabies remains a significant issue and can create a false impression of low burden in rabies endemic settings across Africa and Asia." (lines 524-525). And in the same paragraph further state: "...we also emphasise the pivotal role that genetic data can play in strengthening human rabies surveillance. " (lines 526-528)

6. Author's Contributions: Ensure consistencies in names of authors between what appears in the title and the contributions here. E.g. Gurdeep Jaswant, Criselda Bautista, Brian Ogoti, Joel Changalucha, Julius O. Oyugi, Kathryn Campbell, Mumbua Mutunga, S.M. Thumbi, Katie Hampson, Kirstyn Brunker. For instance, Mwangi SMT was referred to as SM Thumbi in the title, Bautista CT as C Bautista.

We have revised this part and changed the names into full names to be consistent with how it is written in the author's list.


A. Americas
B. Africa
C. Asia
Country
Venezuela
Uruguay
Trinidad & Tobago
Puerto Rico
Peru
Paraguay
Panama
Nicaragua
Mexico
Honduras
Haiti
Guatemala
El Salvador
Ecuador
Dominican Republic
Cuba
Costa Rica
Colombia
Chile
Canada
Brazil
Bolivia
Belize
Argentina
Studies
20
10
0
partial, N=51
WGS, N=1
Gen1, N=50
Gen2, N=1
Gen3, N=0
incountry, N=18
phylodynamic, N=29
Zimbabwe
Zambia
Tanzania
Uganda
Tunisia
Sudan
Spain
South Africa
Sierra Leone
Senegal
Rwanda
Nigeria
Niger
Namibia
Mozambique
Morocco
Mauritania
Mali
Malawi
Madagascar
Liberia
Lesotho
Kenya
Ivory Coast
Guinea
Ghana
Gambia
Gabon
Ethiopia
Egypt
DRC
Chad
CAR
Cameroon
Burkina Faso
Botswana
Benin
Algeria
Studies
10
0
partial, N=97
WGS, N=24
Gen1, N=82
Gen2, N=21
Gen3, N=6
incountry, N=35
phylodynamic, N=53
Vietnam
UAE
Turkey
Thailand
Taiwan
Sri Lanka
South Korea
Saudi Arabia
Russia
Qatar
Philippines
Pakistan
Oman
Nepal
Mongolia
Malaysia
Laos
Kazakhstan
Jordan
Israel
Iraq
Iran
Indonesia
India
Georgia
China
Cambodia
Bhutan
Bangladesh
Azerbaijan
Afghanistan
Studies
50
40
30
20
10
0
partial, N=118
WGS, N=20
Gen1, N=125
Gen2, N=5
Gen3, N=1
incountry, N=113
phylodynamic, N=35

VIRAL SEQUENCING TO INFORM THE GLOBAL ELIMINATION OF DOG-MEDIATED RABIES - **Protocol**

Gurdeep Jaswant[1-4]*, Criselda Bautita[2,5]*, Brian Ogoti[1,6], Joel Changalucha[4,7], Julius O. Oyugi[1,8], Kathryn Campbell[2], Mumbua Mutunga[1,6,], Kirstyn Brunker*[2], S.M. Thumbi*[1,6,7,8,9,10], Katie Hampson*[2]

[1] University of Nairobi, Institute of Tropical and Infectious Diseases, Kenya

[2]School of Biodiversity, One Health & Veterinary Medicine, College of Medical, Veterinary and Life Sciences, University of Glasgow, UK

[3]Tanzania Industrial Research Development Organization, Dar es Salaam, Tanzania

[4]Ifakara Health Institute, Dar es Salaam, Tanzania

[5]Research Institute for Tropical Medicine, Philippines

[6]Center for Epidemiological Modelling and Analysis, University of Nairobi, Nairobi, Kenya

[7]College of Veterinary Medicine & Biomedical Science, Sokoine University of Agriculture, Tanzania

[8]Department of Medical Microbiology, Faculty of Health Sciences, University of Nairobi, Kenya

[9]Institute of Immunology & Infection Research, University of Edinburgh, UK

[10]Paul G Allen School for Global Health, Washington State University, Pullman, US

*Corresponding author: gurdeepjaswant14@gmail.com

Running title / question – How can genetic surveillance support the elimination of rabies?

Link for this
https://docs.google.com/document/d/1S6NHYukEOq6_F5LghQEXm4ja0LyAhhBWlwATdipBTlI/edit?usp=sharing

Abstract

Background

Rabies is a deadly yet neglected infectious disease. Present in almost 150 countries around the world, with most deaths reported in Asia and Africa, rabies is a serious pressing issue worldwide. A global strategy has been initiated with the aim of eliminating human deaths from rabies spread by domestic dogs by 2030 ('Zero by 30'). Genomic surveillance is a tool that can potentially support the 'Zero by 30' strategy.

Methods

The databases PubMed, Google Scholar and Web Of Science will be searched to identify original studies published since the year 2000 with the following search terms 'Rabies AND (genom* OR sequenc* OR phylo* OR molecular) AND (control OR surveillance OR eliminat*)'. Pre-defined inclusion and exclusion criteria will be used to select relevant studies and the selection procedure will be shown by a Preferred Reporting Items for Systematic reviews and Meta-analysis study flow diagram (PRISMA). Data will be extracted including author, year of publication, location of study, study design, sequencing platform, and coverage of genome (whole or partial), type and number of samples sequenced, infected host species, analysis methods, conclusion(s) of the study and any recommendations for control measures or surveillance derived from the genomic data. Data will be summarized in terms of trends in published papers, geographical coverage, sequencing platforms, length, and available genomic data.

Expected output

To get geographical coverage of the country with sequencing data in Africa to see where gaps are to expand the need of genomic surveillance. To give recommendations on how to improve genomic surveillance based on sample types, sequencing platforms, and data management. Key messages from study's conclusion regarding how genomic surveillance provides insights to inform Zero by 30 by drawing the unique message from genomic data.

Registration: This protocol will be submitted to the PROSPERO database for registration.

Keywords: Sequencing, phylogenetic, lyssavirus, molecular techniques

Background

Rabies Virus (RABV) poses the greatest public health threat, causing an estimated 60,000 deaths annually, almost all of which occur in Low- and Middle-Income Countries (LMICs) [1]. RABV is most commonly transmitted through bites from infected hosts in the orders *Chiroptera* and *Carnivora* [2]. Domestic dogs are the main source of transmission to humans, but as a multi-host pathogen, wild carnivores also serve as primary RABV hosts with host-associated variants recorded in certain geographies [3]. For example, wildlife such as raccoons, skunk, and foxes each maintain different RABV variants in localities across

North America [3]. Generally, RABV is referred to according to these host-associated variants (sometimes termed biotype, see definitions introduced in Box 1). Phylogenetic analysis enables further classification of RABV diversity into clades, subclades, and lineages, usually associated with specific geographic areas and/or hosts. The RABV genome is 12 kilobases (kb) in length [4], comprising five genes encoding the nucleoprotein (N), phosphoprotein (P), matrix protein (M), glycoprotein (G) and the large polymerase protein (L) [5]. Like other RNA viruses, RABV exhibits elevated mutation rates because of the absence of proofreading activity in the L protein [6]. Viral sequence data is informative because of these elevated mutation rates, which generate genetic diversity enabling improved tracking of viral spread and understanding of viral dynamics across space and over time.

There is no treatment for rabies once clinical signs begin, but post-exposure prophylaxis (PEP) administered shortly after a rabies exposure is almost 100% effective in preventing the fatal onset of disease [7]. Canine rabies elimination is possible through mass dog vaccination, as demonstrated in Europe, North America, parts of Asia and much of Latin America [8]. Several countries where dog-mediated rabies was previously endemic have now been declared rabies-free (Western Europe, Canada, the USA, and Japan) or are approaching elimination as a result of sustained dog vaccination [8]. According to the World Health Organisation (WHO), to eliminate dog-mediated rabies, vaccination campaigns need to achieve coverage of at least 70% of the dog population and be conducted annually for at least three years [9]. The incidence of rabies in Latin America has declined dramatically over recent decades due to coordinated regional elimination programs underpinned by this approach [8]. In contrast, most LMICs in Asia and Africa have not allocated sufficient budget to control this neglected disease. In these endemic countries, rabies surveillance is typically poor and challenges to rabies control include lack of understanding of dog ownership patterns, dog population sizes and dog accessibility for vaccination as well as cultural practices including dog meat consumption [10]. To address these challenges, international organisations recently joined forces under the United Against Rabies collaboration to advocate for the global goal of ‘Zero by 30’, to end human deaths from dog-mediated rabies by 2030 [11].

Surveillance plays a critical role in the control and elimination of infectious diseases [12]. Surveillance entails the continuous, systematic collection, analysis, interpretation, and timely dissemination of health-related information [13], serving as the foundation for planning, execution and evaluation of public health strategies. For instance, surveillance aids in producing data on the effectiveness of interventions, thus offering valuable insights for decision-making crucial for elimination initiatives like 'Zero by 30' [14]. Increasingly, surveillance involves genetic data, for pathogen diagnosis, for determining risks associated with a pathogen or its susceptibility to drugs, as well as to identify the source of outbreaks and to characterise pathogen spread [12]. Linked with locations, pathogen genetic data have uncovered different

aspects of disease movement, from global migration dynamics to local transmission pathways for pathogens such as Influenza [15,16], Ebola [17], Zika [18], Yellow fever [19,20], Mpox [21–24] and SARS-CoV-2 [25]. Sequencing approaches have potential to enhance rabies surveillance and provide actionable information to inform rabies control programs locally and as part of 'Zero by 30'. For example, viral sequence data can distinguish continuous undetected local circulation from incursions and potentially identify their sources [26]. More generally, sequencing could provide key insights into how rabies circulates within different populations and the processes responsible for RABV maintenance in specific localities [12,27].

Use of pathogen sequence data within surveillance programmes is, however, not yet routine in most LMICs. Constraints include lack of local sequencing capacity, trained personnel and laboratory resources, affected by the costs of and access to reagents and consumables, as well as power supplies and cold chain [17]. Sequencing technologies have become more affordable, and efforts are underway to improve their accessibility [28]. Indeed, growth in sequencing capacity in LMICs during the COVID-19 pandemic provided evidence of the feasibility of scaling up molecular diagnostics, but also highlighted operational challenges. For example, public health laboratories in Nigeria capable of molecular identification of SARS-COV-19 from clinical specimens increased from four to 72 laboratories in 2020 [29]. In this systematic review, our goal will be to examine the current extent of the application of genetic approaches to RABV surveillance globally and how, going forward, these approaches can contribute to the global strategy to eliminate human deaths from dog-mediated rabies.

Methods

Search strategy

A systematic search will be done on PubMed, Web of Science and Google Scholar electronic databases to identify original studies that reported genomic surveillance of rabies to support rabies elimination. Advanced searches with Boolean operators and quotations will be performed using the following key terms: 'rabies AND (genom* OR sequenc* OR phylo* OR molecular) AND (control OR surveillance OR eliminat*)'. Further manual searches will be performed for additional relevant studies.

Selection of studies

Data will be extracted from any design (prospective/ retrospective). Pre-defined inclusion and exclusion criteria will be used to select relevant studies and the selection procedure will be shown by a Preferred Reporting Items for Systematic reviews and Meta-analysis study flow diagram (PRISMA) .

Inclusion and exclusion criteria

To ensure that relevant studies are included the following inclusion criteria will be used for screening: studies must address either canine rabies, human rabies or terrestrial wildlife rabies (i.e. not bat rabies), and use molecular techniques with sequencing data either for diagnosis or surveillance of rabies. We will exclude studies reported as literature reviews without presenting data, studies that are not published in English language, duplicated papers, that do not focus on rabies or include genomic/ sequencing data. We will follow PRISMA (Moher et al. 2009) guidelines to determine the Population, Intervention, Comparison and Outcome of the study (PICO), which for our study covers:

P (Population) = Rabies virus

I (Intervention) = genomic sequencing approaches

C (Comparison) = Known rabies control and prevention measures such as Mass dog vaccination and Post Exposure Prophylaxis (PEP)

O (Outcome) = Primary outcome - rabies control guidance; Secondary outcome – Other message from genomic surveillance

Management of identified articles and Quality assessment

All the articles identified from database searches will be exported for duplicate removal, screening of titles, abstracts and eligibility assessment according to the specified inclusion and exclusion criteria. Two independent reviewers will assess the quality of studies to be included in the systematic review. Any discrepancy observed between reviewers regarding the quality of selected study (s) will be resolved through discussion.

Data extraction and analysis

Data from eligible studies will be extracted into spreadsheets. with the following information , author and year of publication; location of study (country and subnational administrative unit if reported), study design, platform for sequencing, type of samples used (brain, saliva, vaccine or other), species of infected animal host (domestic dog, wildlife or other domestic animal, indicating the species involved), sample size (n),

data analysis methods, conclusion(s) of the study and any control measure derived from the sequencing data.

The extracted data will be used to summarise the number of published papers and trends of time, the geographical coverage of the article the (richness of the genomic data available from different areas) , what were the most commonly used sequencing platforms, and implications of the studies on how genomic data draws insight for rabies control and elimination.

Expected output

To get geographical coverage of the country with sequencing data in Africa so as to see where gaps are to expand the need of genomic surveillance. To give recommendations on how to improve genomic surveillance based on sample types, sequencing platforms, and data management. Key messages from studies conclusion regarding how genomic surveillance provides insights to inform Zero by 30 by drawing the unique message from genomic data.

Ethical approval and consent to participate

Not applicable

Consent for publication

The authors consented for publication

Funding

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Supplementary Table 2. Search terms used to select publications. Three search engines were used to select the articles for this review with search terms as shown.

Database	**Search terms**
PubMed	Rabies AND (genomic OR genome OR sequencing OR sequence OR molecular OR phylogenetic OR phylogeny OR phylogeography OR phylodynamic) AND (control OR surveillance OR elimination OR eliminate) NOT (Bat rabies)
Web of Science	(Rabies AND (genom* OR sequenc* OR molecular OR phylo*) AND (control OR surveillance OR eliminat*)) NOT (Bat rabies)
Google scholar	Rabies AND (genomic OR genome OR sequencing OR sequence OR molecular OR phylogenetic OR phylogeny OR phylogeography OR phylodynamic) AND (control OR surveillance OR elimination OR eliminate)

Supplemetary Table 3. Example of the MeSH term used to select publications in PubMed database search.

Search: **rabies AND genomic AND surveillance** Filters: **from 2000 - 2020**

("rabies"[MeSH Terms] OR "rabies"[All Fields]) AND ("genome"[MeSH Terms] OR "genome"[All Fields] OR "genomes"[All Fields] OR "genome s"[All Fields] OR "genomically"[All Fields] OR "genomics"[MeSH Terms] OR "genomics"[All Fields] OR "genomic"[All Fields]) AND ("epidemiology"[MeSH Subheading] OR "epidemiology"[All Fields] OR "surveillance"[All Fields] OR "epidemiology"[MeSH Terms] OR "surveillance"[All Fields] OR "surveillances"[All Fields] OR "surveilled"[All Fields] OR "surveillance"[All Fields])

Translations

rabies: "rabies"[MeSH Terms] OR "rabies"[All Fields]

genomic: "genome"[MeSH Terms] OR "genome"[All Fields] OR "genomes"[All Fields] OR "genome's"[All Fields] OR "genomically"[All Fields] OR "genomics"[MeSH Terms] OR "genomics"[All Fields] OR "genomic"[All Fields]

surveillance: "epidemiology"[Subheading] OR "epidemiology"[All Fields] OR "surveillance"[All Fields] OR "epidemiology"[MeSH Terms] OR "surveillance"[All Fields] OR "surveillances"[All Fields] OR "surveilled"[All Fields] OR "surveillance"[All Fields]

METHODS

PRISMA reporting

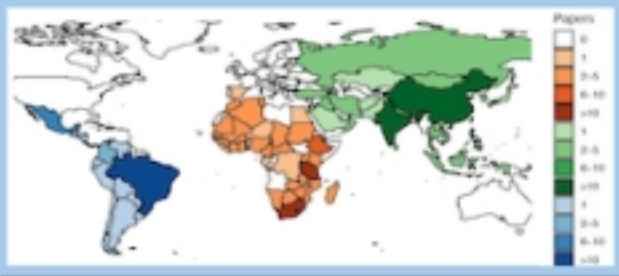
IDENTIFIED
Published 2000-2023
from
Africa, Asia and
Latin America
1581 studies

SCREENED
161 duplicates
1200 excluded

INCLUDED
220 studies

RESULTS

- Dogs are the primary reservoir in these regions
- Cross-species transmission between dog and wildlife
- Lack of standardised nomenclature system



- Phylogenetic analysis resolved origin of most human cases
- Only 29 publications generated whole genome sequences
- Local and long-distance / transboundary transmission identified

CONCLUSION AND RECOMMENDATIONS