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Mosquito-based detection of retroviruses and arboviruses in Senegal: expanding the scope of xenosurveillance

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Abstract

Background Mosquitoes are well-known vectors for arthropod-borne viruses, yet their role as passive carriers of non-arthropod-borne viruses remains underexplored. Xenosurveillance, a method that utilizes blood-feeding arthropods to sample host and pathogen genetic material, has emerged as a valuable tool in viral ecology. In this study, we investigated the viral landscape of blood-fed mosquitoes from Senegal and report the first detection of Jaagsiekte Sheep Retrovirus (JSRV)-related and Enzootic Nasal Tumor Virus 2 (ENTV-2)-related sequences**,** alongside endemic arboviruses. Our study aimed to investigate whether mosquitoes can serve as sentinels for detecting both pathogens and host-derived markers in complex ecosystems.

Methods Mosquitoes were collected between 2016 and 2019 from three ecologically significant regions in Senegal (Louga, Barkedji, and Kedougou). Blood-fed mosquitoes were pooled and subjected to RNA extraction and metagenomic sequencing using Illumina NextSeq550. Sequencing data were analyzed with CZ-ID and BLAST for viral identification. RT-qPCR assays were designed to validate the presence of JSRV-related sequences, targeting conserved regions of the envelope gene and 3' untranslated region. Phylogenetic analysis was conducted using MAFFT and IQ-TREE to compare the detected sequence with global exogenous and endogenous JSRV references.

Results Sequencing revealed a broad viral diversity across mosquito species, including insect-specific viruses, arboviruses (West Nile, Sindbis, Bagaza, Usutu, Barkedji), and two retroviral sequences. A JSRV-related sequence was confirmed in a pool from Barkedji (2019) and clustered phylogenetically with endogenous JSRV**.** A nearly complete ENTV-2 genome, closely related to pathogenic Chinese strains, was recovered from the same pool**.** Other viruses grouped within established African lineages, supporting persistent regional circulation.

Discussion This study presents the first report of retroviral sequences detected in mosquitoes, alongside the identification of actively circulating arboviruses and insect-specific viruses, highlighting the broader potential of mosquitoes

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as environmental sentinels. While mosquitoes are not biological vectors for retroviruses, their ability to capture both host-derived retroviral material and pathogenic viral genomes through bloodmeals reinforces the value of xenosurveillance for monitoring livestock–vector–environment interactions. These findings contribute to broader efforts in integrated disease surveillance and underscore the utility of combining metagenomics with molecular diagnostics to detect diverse viral signals in high-risk ecological settings.

Keywords Xenosurveillance, Jaagsiekte sheep Retrovirus, Enzootic nasal tumor virus 2, Arboviruses, Senegal, Metagenomics, One Health surveillance

Background

The discovery of viruses transmitted by arthropod vectors has transformed our understanding of viral ecology and infectious disease dynamics [1]. Mosquitoes, in particular, play a critical role in transmitting a wide range of pathogens that affect both animal and human populations [2]. This fact has prompted extensive research into the complex interactions between vectors, viruses, and hosts, with a special focus on zoonotic spillover events where pathogens cross species barriers [3]. Advances in next-generation sequencing (NGS) technologies have further accelerated pathogen discovery, especially in the context of vector-borne diseases. NGS enables high-throughput, unbiased sequencing, allowing for the detection of both known and novel viruses in diverse ecological settings [4].

Xenosurveillance, or the sampling of pathogen genetic material from the blood meals of arthropod vectors, has proven effective for detecting viruses circulating in wildlife and domestic animals [5, 6]. When combined with metagenomic sequencing, xenosurveillance offers comprehensive insights into viral diversity in specific environments. This approach not only aids in the discovery of new pathogens but also helps clarify viral transmission ecology, identifying potential vectors and reservoirs for zoonotic diseases [7].

Jaagsiekte sheep retrovirus (JSRV) is a betaretrovirus known to cause ovine pulmonary adenocarcinoma (OPA), a contagious lung cancer in sheep [8]. Transmission occurs primarily via aerosolized droplets, and the virus has been considered tightly restricted to sheep and closely related ruminants. Two forms of JSRV exist: the exogenous, infectious virus (exJSRV), and multiple endogenous variants (enJSRV), which are stably integrated into the host genome. These endogenous forms share high sequence similarity with exJSRV and retain some functional elements, raising interest in their ecological relevance and evolutionary relationship.

Enzootic Nasal Tumor Virus type 2 (ENTV-2) is another betaretrovirus, responsible for enzootic nasal adenocarcinoma (ENA), a transmissible tumor affecting the upper respiratory tract of goats. Like JSRV, ENTV-2 belongs to the genus *Betaretrovirus* and exhibits a similar genomic structure and oncogenic potential, notably through the activity of its envelope (*env*) protein [9]. ENTV-2 has been reported in caprine populations from Asia and Europe, where it has caused localized outbreaks [10]. While ENTV-2 is not known to be transmitted by arthropods, its detection in environmental samples and its genomic similarity to endogenous retroviral elements make it a relevant target for surveillance efforts at the animal–environment interface.

In this study, alongside other arthropod-borne and non-arthropod-borne viruses, we report the first detection of genetic material to both JSRV and ENTV-2 in blood-fed mosquitoes collected in southeastern Senegal, identified through a vector-enabled metagenomic approach. For JSRV, phylogenetic analysis revealed that the sequence is most closely related to enJSRV. While this finding does not indicate active viral replication or vector-borne transmission, it provides indirect evidence of mosquito contact with local ruminant hosts, particularly sheep. The ENTV-2 sequence detected in mosquito bloodmeals showed strong similarity to strains previously characterized in caprine populations affected by ENA, suggesting contact with infected goats in the environment. These detections support the growing utility of xenosurveillance for capturing host-derived genetic signatures, in addition to circulating pathogens.

Moreover, these findings align with emerging ecological observations, such as the recent detection of JSRV RNA in surface water from Tanzanian waterholes during dry seasons [11], which together raise important questions about non-traditional interfaces of viral exposure and environmental persistence in pastoral ecosystems.

By capturing host-derived retroviral sequences through mosquito metagenomics, our work underscores the broader potential of xenosurveillance to advance understanding of host–vector–environment interactions and to support integrated pathogen and exposure monitoring systems at the intersection of animal and public health.

Methods

Study sites

Blood-fed mosquitoes were collected between 2016 and 2019 from three ecologically significant regions in

Senegal, Louga in the northwest, Barkedji in the central Ferlo region, and Kedougou in the southeast, each chosen for their relevance to vector-borne disease dynamics. Louga serves as a transitional zone between agriculture and pastoralism, where fluctuating rainfall and rising temperatures impact livestock and crop production, fostering mosquito-host interactions [12]. Barkedji, centrally located, features pastoral corridors and wildlife reserves, providing a critical interface for zoonotic spillovers [13]. Pastoral activities in the region are marked by seasonal livestock movements, with sheep and goats forming the backbone of herds due to their resilience to water scarcity. Kedougou lies within a tropical forest zone near the Guinean border, where the humid climate supports high mosquito activity, making it a hotspot for pathogen circulation [14, 15]. Although pastoralism is less dominant in this area compared to the Ferlo or Louga regions, Kedougou has become an important destination for herders from northern Senegal due to its abundant pasture and water resources, especially during dry seasons. Herders are drawn to Kedougou for economic opportunities, including livestock markets where animals command attractive prices, enabling them to diversify their income sources [16].

Mosquito collection and sample selection

This study analyzed archived mosquito pools collected biweekly between 2016 and 2019 as part of the national arbovirus surveillance program coordinated by IPD. Mosquitoes were collected using a variety of trapping methods described by Ndiaye et al., including CDC light traps baited with CO_2, placed at both ground and canopy levels [13]. Traps were deployed near livestock enclosures, village edges, and natural water sources in ecologically diverse regions such as Barkedji, Louga, and Kedougou.

Following morphological identification on the field, mosquito pools were archived at the Centre de Recherche sur les Arbovirus (CRORA) biobank [17]. For this study, a subset of pools was pre-selected based on their ability to induce cytopathic effect (CPE) upon inoculation in mosquito and mammal cell culture but had tested negative by indirect immunofluorescence assay (IFA) for common arboviruses [18]. This selection strategy prioritized samples likely to contain uncharacterized or non-target viral agents.

Whole mosquito specimens from the selected pools were homogenized, and total nucleic acids were extracted for metagenomic sequencing to identify both host- and pathogen-derived viral material.

RNA extraction and metagenomic sequencing

RNA extraction was performed using the MagMax Viral/Pathogen Nucleic Acid Isolation Kit and the KingFisher Flex Purification System (Thermo Fisher), following the manufacturer's recommendations. Sequencing was conducted on the extracted RNA using an Illumina-based unbiased approach, as described previously [19]. Briefly, host ribosomal RNA was depleted using specific rRNA-targeting probes [20] and RNase H, and the remaining RNA served as the template for first-strand cDNA synthesis with the SuperScript IV Reverse Transcriptase kit. Double-stranded cDNA was then generated using Klenow fragment DNA polymerase. Libraries were prepared with the Nextera XT DNA Library Preparation kit (Illumina), and sequencing was carried out on the NextSeq 550 system.

The resulting sequences were analyzed using the CZ-ID metagenomics platform (http://czid.org, accessed on 15 March 2023) for viral identification [21]. Default pipeline parameters were used, incorporating quality control steps such as adapter trimming, low-complexity read filtering, and the removal of host-derived reads (sheep, goat, human) as well as common laboratory contaminants. Sequence alignment was performed against curated nucleotide (NT) and protein (NR) databases using Bowtie2 and RAPSearch2, respectively. Reads were assembled into contigs with SPAdes, and taxonomic classification was based on nucleotide and translated alignments, applying identity thresholds of approximately 80–85% for NT and 90–95% for NR, with e-value cutoffs set at $\leq$ 1e-10.

Additionally, the assembled sequences were subjected to BLAST analysis (Basic Local Alignment Search Tool) against the NCBI nucleotide database to confirm viral identity and assess sequence similarity with known viral genomes, facilitating more precise taxonomic classification and strain identification.

Molecular assay development

To confirm the presence of JSRV detected through NGS, we developed two TaqMan-based reverse transcription quantitative PCR (RT-qPCR) assay were developed. These assays utilized primers and a fluorescently labeled probe designed with Primer3 [22], specifically targeting regions of the envelope gene and the 3'untranslated region (3'UTR) based on our assembled JSRV sequences. The designed oligonucleotide sets were rigorously evaluated in silico for specificity using BLAST analysis, ensuring that they would exclusively bind to the target JSRV sequences without cross-reactivity with other viral or host genomes. In addition to the in-house RT-qPCR, we employed the hemi-nested U3hn PCR, a previously

described method targeting the U3 region of the viral long terminal region (LTR), and known for its high sensitivity in field studies [23]. This assay was used to complement and validate the results obtained with our newly developed RT-qPCR assay. All assays were performed on RNA extracted from mosquito pools, providing a robust confirmation framework for the detection of JSRV-related sequences.

Phylogenetic analysis

The consensus data generated during this study were aligned with a representative dataset of available sequences using the multiple sequence alignment tool MAFFT (version 7) [24] with default parameters. The alignments allowed for a comprehensive comparison of the new sequences with known viral genomes, providing a robust framework to establish their evolutionary relationships. The aligned sequences were then used to construct the maximum likelihood (ML) phylogenetic trees, employing IQ-TREE [25], a widely recognized tool for phylogenetic inference. The best-fit substitution models for the analysis were selected using ModelFinder [26], ensuring an accurate representation of the evolutionary processes underlying the data. The phylogenetic trees was visualized using FigTree (version 1.4.4) (http://tree.bio.ed.ac.uk/software/figtree/., accessed on 17 March 2023), a flexible software for exploring and presenting phylogenetic results.

Results

Viral diversity, co-detection patterns and regional variation in mosquito populations

A total of 78 mosquito pools collected between 2016 and 2019 were included in the metagenomic analysis. Although our primary focus was on blood-fed female mosquitoes, some pools also included non-blood-fed females and a few males due to the retrospective nature of the archived material. These pools were derived from diverse mosquito species and sites across Senegal.

Our metagenomic sequencing identified a wide array of viruses across various mosquito species collected from different regions of Senegal between 2016 and 2019 (Table 1). These included both arboviruses and non-arboviruses, demonstrating the remarkable diversity of viral communities harbored by mosquitoes. Notable detections were observed in species such as *Aedes aegypti*, *Aedes vexans*, *Culex quinquefasciatus*, and *Aedes furcifer*, each playing a distinct role in the viral ecosystem. The identified viruses spanned multiple viral species, with significant detections of Pestivirus A, Dezidougou virus, and Culex iflavivirus, reflecting a broad spectrum of viral types circulating in the environment.

To further investigate the taxonomic placement of key arboviruses detected, we conducted phylogenetic analyses on selected sequences, including those corresponding to West Nile virus, Usutu virus, Sindbis virus, Bagaza virus, and Barkedji virus. These reconstructions confirmed that the sequences recovered clustered within known African lineages, supporting local or regional endemicity. Notably, the West Nile virus sequence grouped with lineage 2 strains historically linked to sub-Saharan Africa (Supplementary Fig. 1), while the Usutu virus sequence aligned with an African clade previously identified in the country (Supplementary Fig. 2). The Sindbis virus sequence showed high similarity to other West African isolates within the African lineage (Supplementary Fig. 3), and the Bagaza virus sequence was genetically close to strains circulating in birds in the region. The Barkedji virus, first described in Senegal, also clustered with its original prototype strain (Supplementary Fig. 5).

Co-detections were frequently observed across several mosquito species, suggesting complex viral interactions within vector populations. While multiple viruses were identified in some pooled samples, we refer to these as instances of co-detection rather than confirmed co-infections. Given that each pool may contain multiple mosquitoes, the detected viral sequences could originate from different individuals. As such, these findings reflect aggregate viral presence within the pool and do not indicate concurrent infection of a single mosquito.

The metrics associated with viral identification are provided in Supplementary Table 1.

For example, Pestivirus A, known as bovine viral diarrhea virus type 1 infecting various vertebrates including cattle, sheep, goats, pigs and wild ruminants was widely detected in mosquitoes from multiple regions, including Kedougou and Barkedji, particularly in species like *Aedes unilineatus* and *Aedes furcifer*. Dezidougou virus, another frequently identified virus, was found alongside Aedes vexans iflavivirus in *Aedes vexans* pools from Barkedji. In *Culex quinquefasciatus* from Louga, co-detections were observed, with Culex iflavivirus and Pestivirus H appearing in the same pools as Hubei partiti-like virus and Avian Leukosis virus, highlighting the complexity of viral ecosystems in the region.

These findings underscore the extensive viral diversity present within mosquito populations, with marked regional variation observed across the study sites. Distinct viral profiles were found in Barkedji (51 pools, 1,085 mosquitoes), Louga (19 pools, 192 mosquitoes), and Kedougou (8 pools, 70 mosquitoes), likely influenced by local ecological factors. Barkedji and Louga each harbored 13 unique viral species, while Kedougou, a tropical forest site, had only 3. This may reflect our sampling

Table 1 Mosquito Species and Viruses Identified Across Collection Sites (2016–2019)

Mosquito species	Collection locality	Sex	Date of Collection (Year-Month)	Identified Viruses	Number of pools	Number of individuals	Feeding Status
Aedes aegypti	Louga	Female	2017–10	-	2	2	Fed-blood
		Male		Aedes Albopictus Densovirus/Dezidougou virus	1	1	Unfed
			2017–11	Dezidougou virus	1	2	Unfed
				Pestivirus A	1	1	Unfed
	Kedougou	Female	2019–10	Pestivirus A	1	2	Unfed
				Pestivirus A/Taesano Aedes Virus	1	2	Fed-blood
				Taesano Aedes Virus	1	4	Unfed
Aedes centropunctatus	Kedougou	Female	2019–10	Pestivirus A	1	1	Unfed
Aedes dalzieli	Barkedji	Female	2017–08	-	1	5	Unfed
			2019–09	-	1	1	Unfed
	Kedougou		2019–10	Pestivirus A	2	58	Unfed
Aedes furcifer	Kedougou	Female	2019–10	Pestivirus A/Mosquito Densovirus BR/07	1	2	Fed-blood
Aedes ochraceus	Barkedji	Female	2017–08	-	1	5	Unfed
Aedes sudanensis	Barkedji	Female	2017–08	-	1	1	Unfed
			2019–09	Dezidougou Virus/Daezongdong Virus	1	1	Unfed
Aedes taylori	Kedougou	Female	2019–10	-	1	1	Fed-blood
Aedes unilineatus	**Barkedji**	**Female**	**2019–09**	**Jaagsiekte Sheep Retrovirus/Enzootic Nasal Tumor Virus type 2**/Dezidougou Virus	**1**	**3**	**Fed-blood**
				Pestivirus A	1	2	Fed-blood
Aedes vexans	Barkedji	Female	2016–09	-	6	213	Unfed
			2017–08	-	4	114	Unfed
				Dezidougou virus/Aedes vexans iflavivirus	1	40	Unfed
			2019–08	-	1	50	Unfed
				Dezidougou Virus	4	200	Unfed
			2019–09	-	1	1	Unfed
				Aedes Vexans iflavivirus	1	3	Unfed
				Aedes Vexans iflavivirus/Dezidougou Virus	1	6	Unfed
				Dezidougou Virus	3	71	Fed-blood
Anopheles pharaonsis	Barkedji	Female	2017–08	-	1	3	Unfed
		Male		-	1	1	Unfed
Culex quinquefasciatus	Louga	Female	2017–10	Culex iflavivirus/Pestivirus H	1	5	Fed-blood
				Hubei partiti-like Virus	1	1	Unfed
				Hubei partiti-like Virus/Avian Leukosis Virus/Yongsan iflavivirus/Culex iflavivirus	1	2	Fed-blood
			2017–11	-	4	66	Fed-blood
				Culex iflavivirus/Avian leukosis Virus	1	39	Fed-blood
		Male	2017–11	-	1	35	Unfed
				Aedes Albopictus Densovirus	1	5	Unfed
				Culex flavivirus/Culex Densovirus/Mosquito Densovirus BR/07	1	16	Unfed
				Pestivirus H/Hubei mosquito virus/Culex iflavivirus	1	15	Unfed

Table 1 (continued)

Mosquito species	Collection locality	Sex	Date of Collection (Year-Month)	Identified Viruses	Number of pools	Number of individuals	Feeding Status
Culex tritaeniorhyncus	Louga	Female	2017–11	-	2	2	Unfed
Culex antennatus	Barkedji	Female	2017–08	-	1	1	Unfed
Culex ethiopicus	Barkedji	Female	2016–09	-	1	11	-
			2019–09	-	1	25	-
				Pestivirus A/Dezidougou Virus	1	37	-
Culex neavei	Barkedji	Female	2019–10	Barkedji Virus/Bagaza Virus/Sindbis Virus/West-Nile Virus	1	50	-
				Barkedji Virus/Bagaza Virus/Usutu Virus	1	50	-
Culex perfuscus	Barkedji	Female	2016–09	-	1	4	-
Culex poicilipes	Barkedji	Female	2017–08	-	2	3	-
				-	1	6	-
			2019–09	-	1	33	-
				Dezidougou Virus/Aedes vexans iflavivirus	1	50	-
				Dezidougou Virus/Daezongdong Virus	1	50	-
				Pestivirus A	2	37	Fed-blood
Culex quinquefasciatus	Barkedji	Female	2019–09	Aedes Pseudocutellaris Reovirus/Pestivirus A	1	1	Fed-blood
				Dezidougou Virus	1	1	-
				Pestivirus A	1	1	Fed-blood
Culex tigripes	Barkedji	Female	2017–08	-	1	1	-
Phlebotom sp	Barkedji	Female	2019–10	-	1	4	-

strategy, which focused on pools that induced cytopathic effects in mosquito or mammalian cell cultures but tested negative for common arboviruses, enhancing the detection of uncharacterized or non-target viruses. Such variation points to the critical role of regional climate, host availability, and habitat conditions in shaping mosquito-associated viral communities.

Notably, co-detections within the same mosquito pools were more common in samples from Louga and Barkedji, suggesting that these regions may be hotspots for viral transmission and co-circulation. While we cannot infer true co-infections at the individual mosquito level due to the use of pooled samples, these patterns may reflect overlapping ecological niches or shared host exposure, facilitating the simultaneous presence of multiple viral species in the same sampling units.

Detection of retroviruses

Among the various viruses identified, our analysis revealed the first detection of JSRV and ENTV-2 genetic material in mosquitoes. From a blood-fed mosquito pool collected in Barkedji in 2019, we recovered a partial genome sequence (about 600 base pairs in length) encompassing the terminal region of the *Env* gene and 3'UTR of JSRV. Based on these findings, we developed

Table 2 Jaagsiekte sheep retrovirus-targeted RT-qPCR assays designed during the study

RT-PCR set 1	Enveloppe gene	JaagsiekteV_Left primer_1	CGGTTCTGACTGTTGTGCTT
		JaagsiekteV_Right primer_1	CGCAGCTCCCCTCTCTTTAT
		JaagsiekteV_Probe_1	FAM—AACATGTTGCAACACCGACA—BBQ
RT-PCR set 2	3'untranslated region	JaagsiekteV_Left primer_2	CCTAAGCTCCCTGTCCCG
		JaagsiekteV_Right primer_2	GCCTTCCTTTATTGTGCTGC
		JaagsiekteV_Probe_2	FAM—TGTGAATGTCAGAAGTCACGT—BBQ

two sets of molecular assays to confirm the taxonomic identity of JSRV (Table 2).

To further support these results, we also employed the previously validated U3hn PCR to complement our in-house assays. The U3hn PCR served as an independent molecular confirmation of JSRV presence, particularly useful in low-viral-load samples.

RT-qPCR and U3hn PCR were both performed on the mosquito pool in which JSRV was initially detected, along with all nine additionally blood-fed pools with no prior evidence of infection. The assays confirmed JSRV presence in the original mosquito pool (Supplementary Table 2), though follow-up sequencing did not yield additional coverage of the viral genome.

Given that a nearly complete genome was obtained for ENTV-2 (about 5,700 base pairs in length) from the same mosquito pool, no further confirmatory or functional analyses were conducted for this virus, and its detection was interpreted solely based on the metagenomic and phylogenetic evidence. Nonetheless, the successful recovery of such a long fragment underscores the capacity of xenosurveillance to reveal near-complete genomes of host-associated retroviruses from mosquito-derived material.

Phylogenetic analysis of Jaagsiekte sheep retrovirus and Enzootic Nasal Tumor Virus type 2

To further characterize the JSRV-related sequence detected in mosquitoes, we performed a phylogenetic analysis using a multiple sequence alignment covering a region of the partial Env gene and the LTR. This alignment included reference betaretroviruses, with particular emphasis on both exJSRV and enJSRV sequences. A ML phylogenetic tree was then constructed to determine the evolutionary placement of the mosquito-derived sequence.

The resulting tree (Fig. 1) revealed that the sequence clusters closely with enJSRV strains integrated in the sheep genome, rather than with known exJSRV. High bootstrap support (> 90%) on key branches further confirms the robustness of these evolutionary relationships.

Strikingly, from the same pool, a nearly complete sequence showed high similarity to ENTV-2. Phylogenetically, it formed a strongly supported subclade with the Chinese strain GDQY2017 (accession number: MK164396), previously isolated from clinically affected goats in Guangdong province in 2017 [27], and four other recent ENTV-2 isolates from China: OR669623.1 (2022), MN564749.1 (2019), PP682590.1 (2023), and KU258873.1 (2013). The short branch length between the mosquito-derived sequence and GDQY2017 highlights their close genetic relationship, suggesting a potential link to pathogenic strains associated with active disease (Fig. 2).

Together, these results suggest that this mosquito pool of *Aedes unilineatus* likely contained individuals that had fed on different ruminant hosts, some on sheep, others on goats, resulting in the co-detection of retroviral genomic fragments from distinct sources: an endogenous ovine retrovirus and a pathogenic caprine retrovirus.

The phylogenetic proximity of the ENTV-2-like sequence to East Asian strains is intriguing, particularly given the West African context of the sampling. It raises the possibility of an unrecognized introduction and establishment of ENTV-2–like viruses in African goat populations, or a case of evolutionary convergence among betaretroviruses in geographically distant but ecologically similar ruminant systems.

These findings highlight the power of xenosurveillance not only to detect viral diversity, but also to generate evolutionary hypotheses regarding the presence, origin, and movement of host-associated viruses, including those not known to be vector-borne.

Discussion

This study provides significant insights into the viral ecosystems within mosquito populations in Senegal, identifying a diverse array of viruses. These included not only non-arboviruses such as Pestivirus A**,** Dezidougou virus**,** and Culex iflavivirus**,** but also several medically and ecologically important arboviruses**,** including West Nile virus**,** Usutu virus**,** Bagaza virus**,** Sindbis virus**,** and Barkedji virus. The extensive detection of these viruses across various mosquito species underscores the complexity of viral interactions within vector populations and the wide range of host–vector–pathogen interfaces that can be captured through xenosurveillance. Phylogenetic analyses confirmed the identity of these arboviruses, with most sequences clustering within known African lineages, consistent with patterns of enzootic circulation in the region. In the context of this approach, platforms like CZ-ID provide a standardized method for detecting viral sequences in blood-fed mosquitoes. However, reliance on default parameters may reduce sensitivity to highly divergent or novel viruses, particularly in ecologically rich settings, potentially leading to an underestimation of viral diversity, a limitation we acknowledge in our interpretation. These findings highlight the critical role that mosquitoes play, not only as vectors of well-established arboviruses but also as reservoirs or passive carriers of non-arboviral pathogens [6]. This emphasizes the need for broader surveillance efforts to understand the contributions of mosquito populations to viral ecology and pathogen transmission.

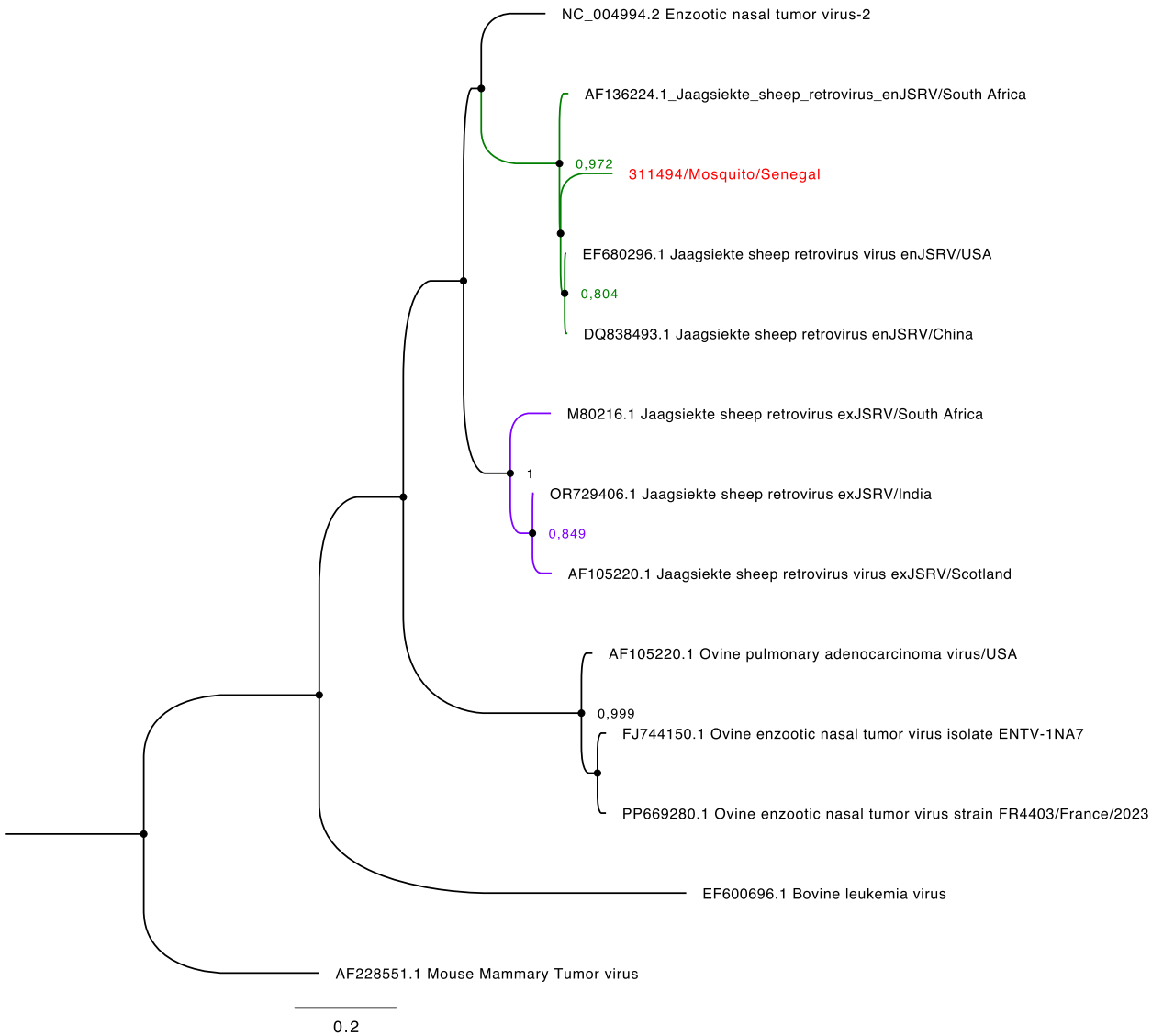

Fig. 1 Phylogenetic analysis of Jaagsiekte Sheep Retrovirus (JSRV)-related sequence detected in mosquitoes from Senegal. Maximum likelihood tree constructed from partial Env–LTR sequences, including the mosquito-derived sequence and representative exogenous (exJSRV, shown with purple branches), endogenous (enJSRV, shown with green branches), and outgroup betaretroviruses (shown with black branches). The Senegalese JSRV sequence clusters closely with known endogenous JSRVs, supporting its origin from host genomic material rather than an exogenous infectious strain. Bootstrap values (> 0.75) are displayed for key nodes to reflect the robustness of the analysis

The detection of JSRV- and ENTV-2-related genetic material in mosquitoes through unbiased metagenomic sequencing adds a novel dimension to vector-based surveillance. Phylogenetic analysis indicates that the detected JSRV sequence is most closely related to enJSRV, non-infectious retroviral elements integrated into the sheep genome. While this does not suggest active viral replication or vector-borne transmission, it provides indirect molecular evidence of mosquito feeding on local ruminant hosts, particularly plausible in Barkedji, a pastoral region characterized by seasonal livestock movements and a high density of sheep and goats. From the same pool, the mosquito-derived ENTV-2 sequence grouped into a well-supported clade alongside several ENTV-2 strains previously detected in clinically infected goats in China, notably including the GDQY2017 strain, which has been associated with disease outbreaks [27]. These findings further highlight the value of xenosurveillance and the potential of mosquitoes to serve as biological samplers, capturing host-derived genetic material through bloodmeals that reflects both infection and exposure in animal and human populations.

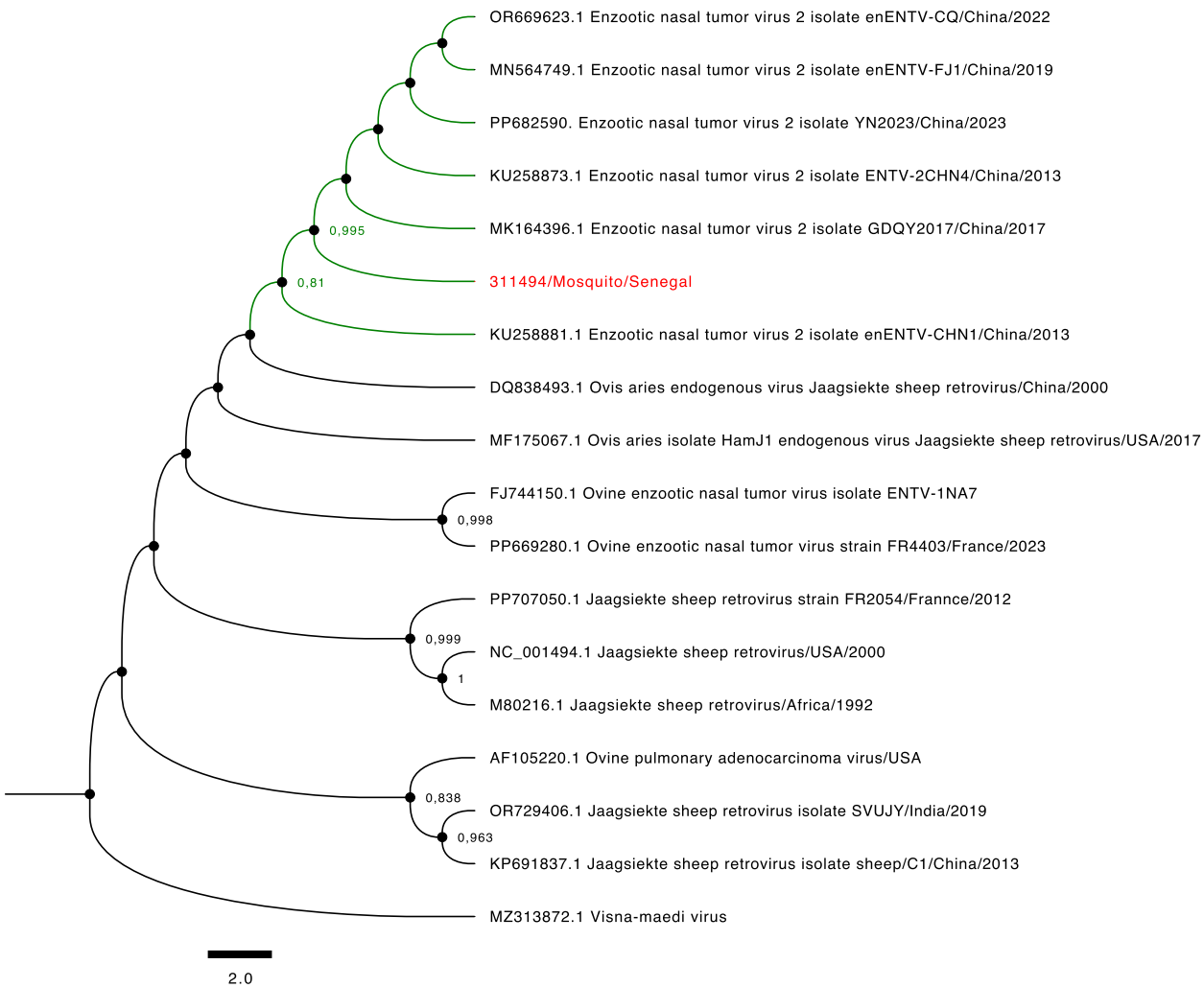

Fig. 2 Phylogenetic analysis of Enzootic Nasal Tumor Virus type 2 (ENTV-2)-related sequence detected in mosquitoes from Senegal. Maximum likelihood tree constructed from nearly complete genome, including the mosquito-derived sequence, some representative ENTV-2, and other betaretroviruses (shown with black branches). The Senegalese ENTV-2 sequence clusters closely with a group of ENTV-2 strains previously associated with clinical disease in goats, suggesting strong genetic similarity and a potential link to pathogenic caprine retroviruses. Bootstrap values (> 0.75) are displayed for key nodes to reflect the robustness of the analysis

The identification of both enJSRV- and ENTV-2-like sequences in the same mosquito pool suggests that mosquitoes can acquire retroviral material from different ruminant species during mixed or sequential bloodmeals. This dual detection underscores the role of mosquitoes as biological samplers of the vertebrate virome, capturing signals not only of classical arbovirus infections but also of host-associated, non-vector-borne retroviruses. The detection of these retroviral elements aligns with prior studies in West Africa and East Africa reporting JSRV-related or ovine retrovirus sequences in human and environmental samples [28–30]. Although the biological relevance of such findings remains unclear, they collectively point to the underappreciated ecological footprint of endogenous and exogenous retroviruses beyond their classical host niches. Further investigation is warranted to better understand the dynamics of retroviral circulation, expression, and detectability in livestock and vector populations. Previous studies in Senegal have reported the presence of ovine betaretroviruses, such as ENTV, directly in infected tissues [31]. By expanding this lens to vector-based environmental surveillance, our findings underscore the potential of mosquitoes to act as passive carriers and ecological sentinels, capturing signals of both active infections and host genomic signatures that may otherwise go undetected.

The utility of xenosurveillance as a tool for pathogen detection is further exemplified in this study. By

analyzing viral genetic material obtained from blood-feeding mosquitoes, we were able to identify viruses circulating within local wildlife and domestic animal populations without the need for direct mammalian sampling. This method offers a less invasive and more practical approach to surveillance, particularly in remote or logistically challenging regions. Xenosurveillance not only expands our understanding of viral geographic distribution and host interactions but also enhances the early detection of emerging zoonotic threats [32]. This approach is especially relevant in areas with high biodiversity and frequent interactions between wildlife, domestic animals, and humans, such as Senegal.

Beyond its contributions to pathogen discovery, this study emphasizes the critical role of molecular diagnostics in enhancing viral surveillance. The development of specific molecular assays targeting JSRV-related sequences, informed by NGS data, was essential to confirm its presence in mosquito samples, particularly given the short genomic fragment retrieved from metagenomic sequencing. In contrast, no complementary PCR assay was performed for ENTV-2, for which a nearly complete genome was recovered. This decision reflects both the robustness of the metagenomic evidence and the absence of prior laboratory manipulation or reference material for this caprine retrovirus in our setting. This integration of high-throughput sequencing with targeted molecular tools exemplifies a robust approach not only for pathogen detection but also for identifying host-derived genetic signatures within vector populations. It also underscores the importance of adapting diagnostic approaches to capture both arboviral and non-arboviral elements, thereby expanding the scope of mosquito-based surveillance beyond classical vector-borne disease paradigms [33]. The implications of this study extend to public health preparedness, as the early detection of emerging viral threats can inform targeted interventions and outbreak prevention strategies.

Ultimately, this work highlights the transformative potential of integrating xenosurveillance with next-generation sequencing and molecular diagnostics to enhance our understanding of virus-vector-host interactions. By detecting host-derived endogenous and exogenous retroviral sequences, dual-host arboviruses, and insect-specific viruses in mosquito bloodmeals, xenosurveillance can provide insight into both pathogen presence and host exposure, capturing ecological contact patterns that extend beyond classical infection-based surveillance. This approach offers a scalable and minimally invasive tool for monitoring livestock health and supporting early-warning systems at the intersection of animal and public health, particularly in pastoral settings where direct sampling remains a challenge. As global health systems move toward integrated One Health frameworks, mosquito-based xenosurveillance offers a unique opportunity to strengthen disease surveillance across species and environments.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s42522-025-00155-7.

Supplementary Material 1.
Supplementary Material 2.
Supplementary Material 3.
Supplementary Material 4.
Supplementary Material 5.
Supplementary Material 6.
Supplementary Material 7.

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Authors' contributions
M.H.D.N., M.D., S.S., M.K., M.M., S.M.B.S.D., S.F.M.D. and M.M.D. contributed to the laboratory experiments; E.H.N., B.D., D.D., N.M.S., F.A.S. and A.G. contributed to the field sampling and species identification; M.H.D.N., A.G. and M.M.D. designed the study; M.H.D.N., E.H.N., A.G. and M.M.D. contributed to the data analysis and curation; A.A.S., O.F., N.D., S.C.W., Oum.F., Maw.D., G.F. and M.M.D. provided administrative and logistical support; M.H.D.N., E.H.N., A.G. and M.M.D. drafted the manuscript. All authors reviewed and approved the final version of the manuscript.

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Data availability
All data generated or analyzed in this study are provided within the article and its supplementary materials, while the genomic data is publicly accessible in the NCBI repository.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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