

<https://doi.org/10.1038/s41522-024-00613-6>

Viruses in the female lower reproductive tract: a systematic descriptive review of metagenomic investigations

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The lower female reproductive tract (FRT) hosts a complex microbial environment, including eukaryotic and prokaryotic viruses (the virome), whose roles in health and disease are not fully understood. This review consolidates findings on FRT virome composition, revealing the presence of various viral families and noting significant gaps in knowledge. Understanding interactions between the virome, microbiome, and immune system will provide novel insights for preventing and managing lower genital tract disorders.

The lower portion of the female reproductive tract (FRT), the vagina and cervix, hosts a complex microbial environment composed of bacteria (bacteriome), archaea (archaeome), fungi (fungome), and viruses (virome). Their individual metabolisms and interactions contribute to the composition of the host local environment and influence the manifestation of gynecological disorders¹. The great majority of FRT microbial investigations have focused on the bacteriome and used the term “microbiome” as a substitute for bacteriome. We follow this convention in the present report. The composition of the FRT microbiome has been shown to influence susceptibility to exogenous pathogens^{2,3} as well as to adverse reproductive outcomes such as preterm birth, miscarriage, and infertility^{2,3}. The cervicovaginal microbiome likely also influences defense against malignant transformation at that site^{4,5}.

Much less studied is the composition of the lower FRT virome. Unlike the microbiome, where analysis of different regions of the gene coding for bacterial 16S ribosomal RNA can provide a comprehensive description of the bacterial composition present in any individual, viral detection is exceedingly more complex. Recent advances in next-generation sequencing (NGS) technologies have propelled studies to identify components of the cervicovaginal virome worldwide⁶.

Despite these technological advancements, our understanding of FRT virome composition and its influence on cervicovaginal and reproductive health remains limited. Most studies have focused on the detection of select components of the virome, rather than providing a comprehensive analysis of total virus composition. Individual variability in both microbiome and virome composition in the FRT occurs due to a multitude of factors such as ethnicity, genetic background, environmental exposures, physiological status, and lifestyle variables including diet, hygiene practices, smoking and contraceptive methods⁶. This variability underscores the necessity to

contextualize virome data within the broader spectrum of individual and environmental differences which directly affect FRT well-being.

The present systematic review aims to consolidate current knowledge of the complexity and variability of the FRT virome by providing a detailed overview of the available literature. Lastly, we seek to highlight key areas for future research.

Results

Literature search and study selection

The initial search identified 7495 records, of which 5559 duplicates were removed, leaving 1936 records for title and abstract screening. 1793 studies were excluded due to lack of viral data, involved non-human species, or did not focus on the FRT. Following a full-text assessment of 143 studies, 29 were excluded due to inappropriate study design or population, 65 for conducting only 16S bacterial ribosomal RNA sequencing, and 15 for being non-original articles. Consequently, 34 studies were included in the final analysis (Supplementary Note 2)⁷⁻⁴⁰. A flowchart of the literature search process is provided in Supplementary Fig. 1.

Metadata Analysis

Among the 34 studies analyzed, cross-sectional designs predominated in 22 (64.7%), while only 12 studies (35.3%) employed a longitudinal design. Half of the studies (17) focused on reproductive-age cohorts, with the remainder involving mixed age groups (11 studies), adolescents (2 studies), pre-menopausal/menopausal women (2 studies), and 2 studies that did not specify the cohort age.

Various enrichment methods were employed, with targeted sequence capture and rolling circle amplification being the most common (14.7% and

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11.7%, respectively). Three studies used other distinct enrichment methods, while 22 studies (67.7%) did not apply any enrichment method.

The majority of studies utilized silica membrane-based extraction methods (73.5%), followed by magnetic bead-based techniques (14.7%). Additionally, three studies used solvent-based methods, and one study employed a column-based method. DNA was the primary nucleic acid extracted in most studies (67.6%), with 10 studies (29.4%) extracting both DNA and RNA, and only one study focusing solely on RNA. Among the included studies, 8 (23.5%) incorporated an internal control to monitor potential background contamination, ensuring that viral contamination of reagents, equipment, and personnel was ruled out, while 26 (76.4%) did not specify any controls.

Illumina platforms were predominantly used for sequencing (88.2%), followed by BGI (5.9%). One study utilized Ion Torrent (2.9%), and another employed multiple sequencing platforms. *Lactobacillus spp.* analysis was performed in 29 studies (87.9%), with *Lactobacillus* predominance observed in 25 of these (86.2%).

The majority of studies made their metagenomic data publicly accessible, with 27 studies (79.5%) depositing data in ENA/SRA, 3 (8.8%) in CNGBdb, and 1 (2.9%) in EBI; however, 3 studies (8.8%) did not provide data availability. An overview of the key aspects and metadata from each included study is provided in Fig. 1.

Geographical distribution and sample characteristics

The geographical distribution of the studies was diverse, with a concentration in the Americas (35.3%) and the Western Pacific region (29.4%). European and African regions were represented by 5 studies each (14.7%), while 2 studies (5.9%) were conducted in the South-East Asian region.

Sample sizes and types varied significantly, with a total of 3477 samples sequenced across 13 different sample types. The most common sample types were cervical swabs/mucus (1378 samples), cervicovaginal swabs/secretion (734 samples), and vaginal swabs/secretion (629 samples). The distribution of sample types and their respective countries is illustrated in Fig. 2.

Study conditions and cohort control group usage

Regarding cohort control groups, 15 of 16 studies from women with a disorder or infection compared virome findings with healthy women, while

only 3 of 17 studies on healthy pregnant or nonpregnant women or women undergoing a cycle of in vitro fertilization examined a control group. Among studies on women with vaginal dysbiosis (atypical vaginal microbiota), neoplasia, or viral infections, 8 studies (23.5%) investigated human papillomavirus (HPV)-related lesions, and individual studies addressed conditions such as vaginitis, candidiasis, HIV, and malignancy. None of the studies comparing the virome in women with a medical disorder with that of control women identified a viral signature that defined a specific condition. The only differences noted were a variation in the number of viruses identified, either an increase or a decrease depending on the specific study evaluated.

Viral families reported

A total of 79 different viral families were identified across the studies, comprising 55 eukaryotic and 24 prokaryotic viral families. The most frequently reported eukaryotic families were *Papillomaviridae* (97%), *Anelloviridae* (55.9%), and *Orthoherpesviridae* (47%). Among prokaryotic viruses, *Siphoviridae* (41%), *Myoviridae* (38%), and *Podoviridae* (29.4%) were the most common bacteriophage families identified. Notably, 67.6% of the studies reported the detection of viruses that could not be classified utilizing current criteria. The distribution of viral families across the 34 studies is depicted in Fig. 3.

Variability in virome composition across studies may reflect differences in study populations, methodologies, or sequencing techniques. To specifically address the virome in healthy women, we analyzed the 14 studies focused on healthy women. In this subgroup, *Papillomaviridae* was the most prevalent viral family (78.6%), followed by unclassified viruses (64.3%), and *Anelloviridae*, *Orthoherpesviridae*, and *Siphoviridae*, each with a reported prevalence of 42.9% (Fig. 4).

Discussion

Multiple viral species are clearly present in the lower FRT of most healthy women as well as in those with a variety of disorders. Their detection was certainly anticipated, based on the ubiquitous presence of prokaryotic viruses and eukaryotic viruses at every body site that has been investigated⁴¹. However, the composition of the virome can vary significantly across studies, even when similar sample types or clinical conditions are considered.

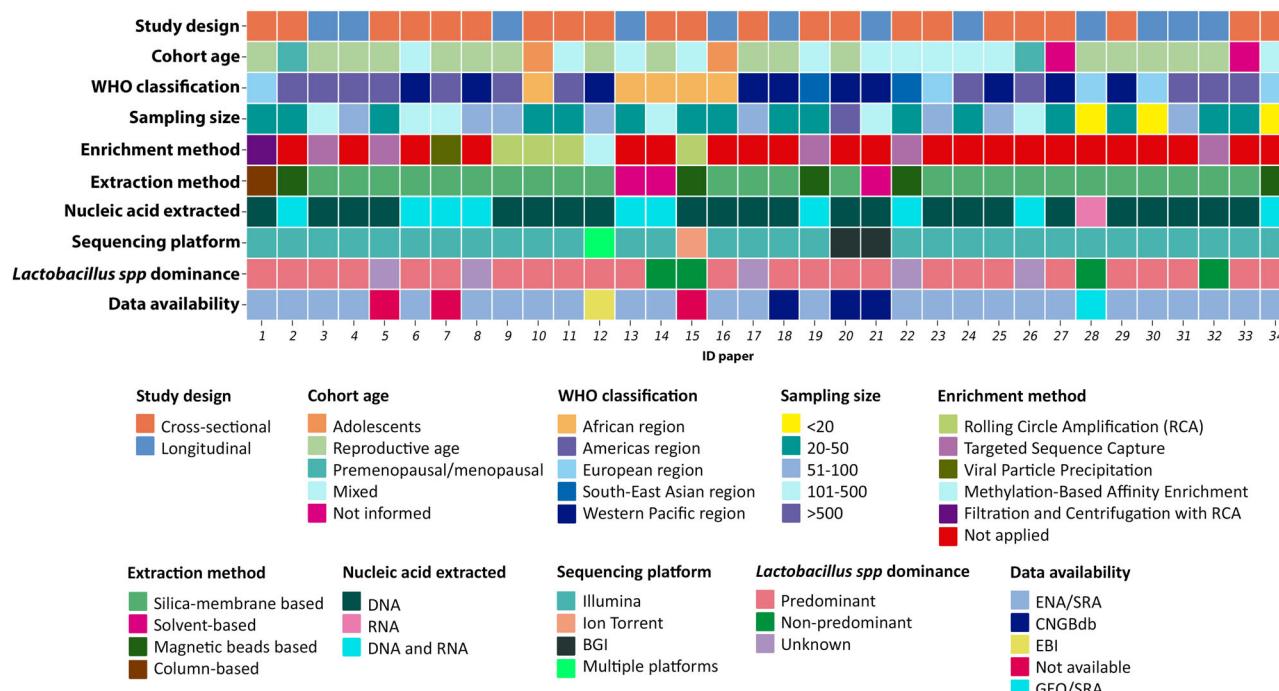


Fig. 1 | Overview of the key aspects and metadata of 34 included studies of the female reproductive tract virome. Each study is represented by a column, with colors indicating specific characteristics and methods used across the studies.

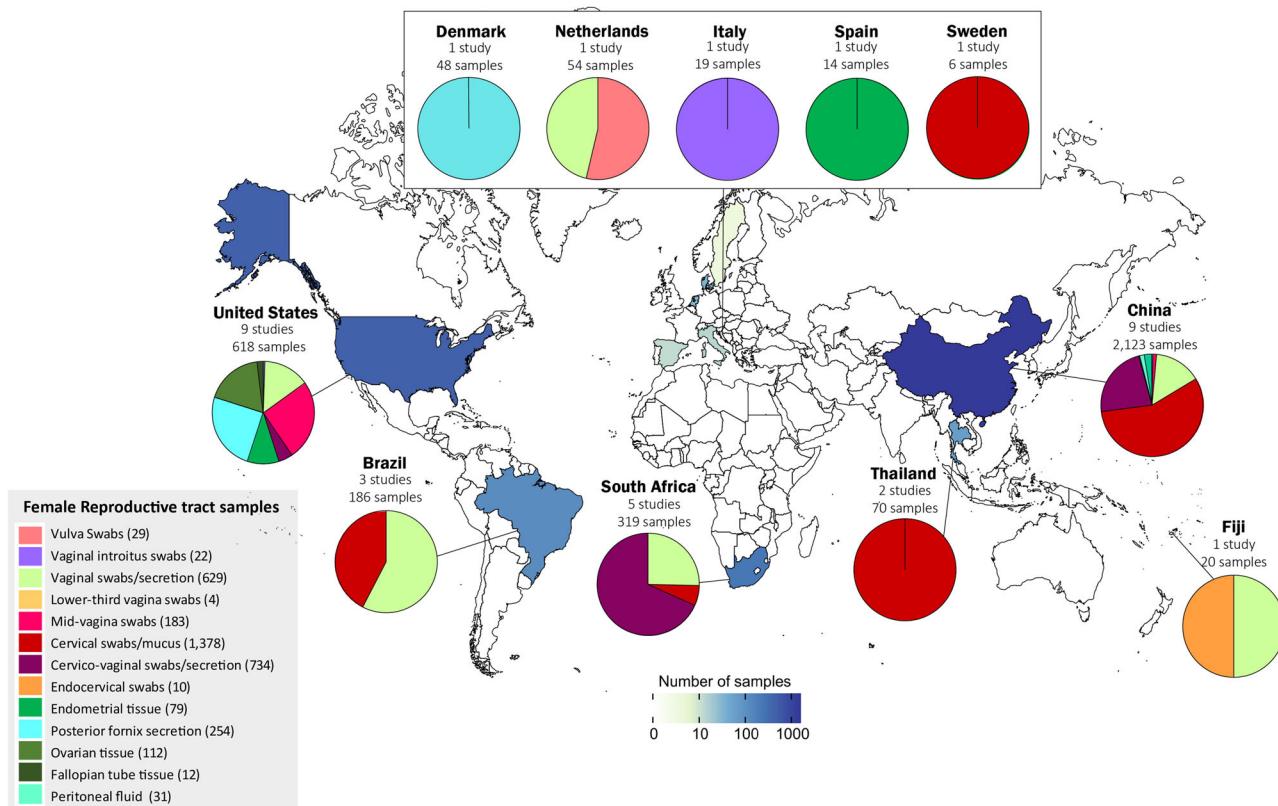


Fig. 2 | Geographical distribution and types of female reproductive tract samples analyzed in the included studies. The map highlights the number of studies and samples collected across different countries, with pie charts indicating the proportion of various sample types used.

This variation may be attributed to methodological differences, including variations in extraction methods, the use of enrichment or purification techniques, and bacteriophages bioinformatics pipelines. Enrichment-based protocols offer the advantage of increasing sensitivity for detecting low-abundance viruses, enabling more targeted analyses. These approaches, though, have limitations, such as the potential loss of non-target viruses, the preferential detection of specific viral populations (e.g., circular viruses when using RCA), and the exclusion of integrated viruses, particularly when host genomes are depleted⁴². Additionally, during material extraction, the use of DNase and filtration can inadvertently remove small viral genomes or integrated viruses and filter out larger viruses, respectively^{34,43}. Another concern is contamination, which can arise from environmental sources, other body sites, or experimental operations³⁸. Moreover, sequence assignments can differ depending on the use of updated reference databases and catalogues across studies, impacting virome characterization and diversity analysis⁴⁴.

It is likely that the continued evolution of more sophisticated protocols for viral detection and quantification will lead to an enhanced identification of different species and an improved understanding of their role in FRT health. A major challenge is to analyze associations between the presence and/or titer of specific viruses as a means to predict the onset of pathological conditions or even as targets for early intervention.

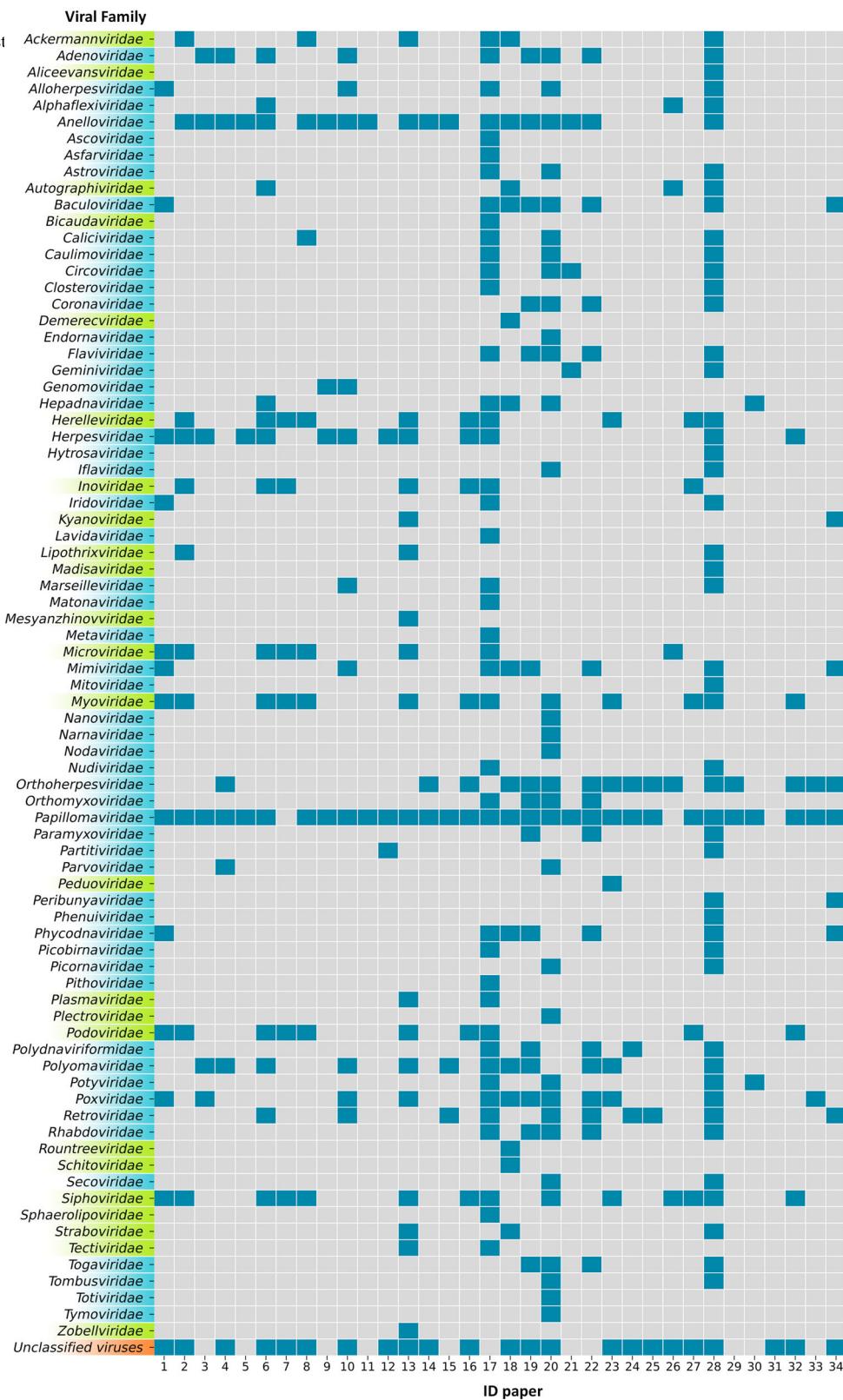
For example, it has been established that the bacterial composition of the vagina in reproductive-age women has a major influence on the occurrence of symptomatic vulvovaginal disorders such as bacterial vaginosis (BV), vulvovaginal candidiasis, aerobic vaginitis, among others⁴⁵. Emerging evidence suggests that transkingdom interactions between bacteriophages and bacterial species may contribute to these conditions, particularly in bacterial vaginosis, where such dynamics could drive alterations in microbial communities and influence genital inflammation^{7,8}. Bacteria-bacteriophage transkingdom analysis showed that women with genital inflammation and BV-associated bacteria (such as *Mobiluncus*, *Mycoplasma*, *Gardnerella*,

Prevotella, and *Sneathia*) shared similar phage interaction profiles⁸. A study by Madere et al.¹¹ demonstrated that bacteriophages present in vaginal swabs cluster into two distinct bacteriophage community groups based on their diversity. These groups were correlated with bacterial communities dominated by *Lactobacillus* spp. (low-diversity bacteriophages) and those dominated by non-*Lactobacillus* spp. (high-diversity bacteriophages), which included potentially harmful bacteria such as *Gardnerella vaginalis* spp. and *Prevotella* spp¹¹. However, it remains unclear whether bacteriophage interactions with individual bacterial species in the FRT contribute to pathological changes. Some studies suggest that bacteriophage composition reflects differences between low and high diversity bacteriomes, but the exploration of these populations and interactions remains limited, and whether demographic or behavioral characteristics (e.g., a woman's smoking status) influence this interaction^{7,11,21}. Furthermore, it needs to be investigated whether exogenous administration of selective bacteriophages can prevent or reverse symptomatic disorders such as bacterial vaginosis.

The human body harbors a remarkable diversity of viral communities that play significant roles in shaping the host physiology and immune phenotype. Chronic systemic viruses, such as herpesviruses, polyomaviruses, anelloviruses, adenoviruses, and papillomaviruses, are known to interact continuously with the immune system¹⁹. These interactions can lead to immune activation through the recognition of pathogen-associated molecular patterns and antigens produced during viral replication or reactivation. As such, commensal viruses may contribute to upregulated innate immunity, providing protection against pathogenic viral or bacterial infections^{11,46,47}. However, in certain contexts, such as in disease states or immunodeficiencies, the virome's interaction with the immune system can be harmful⁶.

In the context of the vaginal virome, immunodeficiency can allow the establishment of viral infections that are less frequently detected in immunocompetent hosts, including genomoviruses (often associated with fungal co-infections), herpesviruses and a higher diversity of papillomaviruses³⁹. The common presence of low-virulence viruses

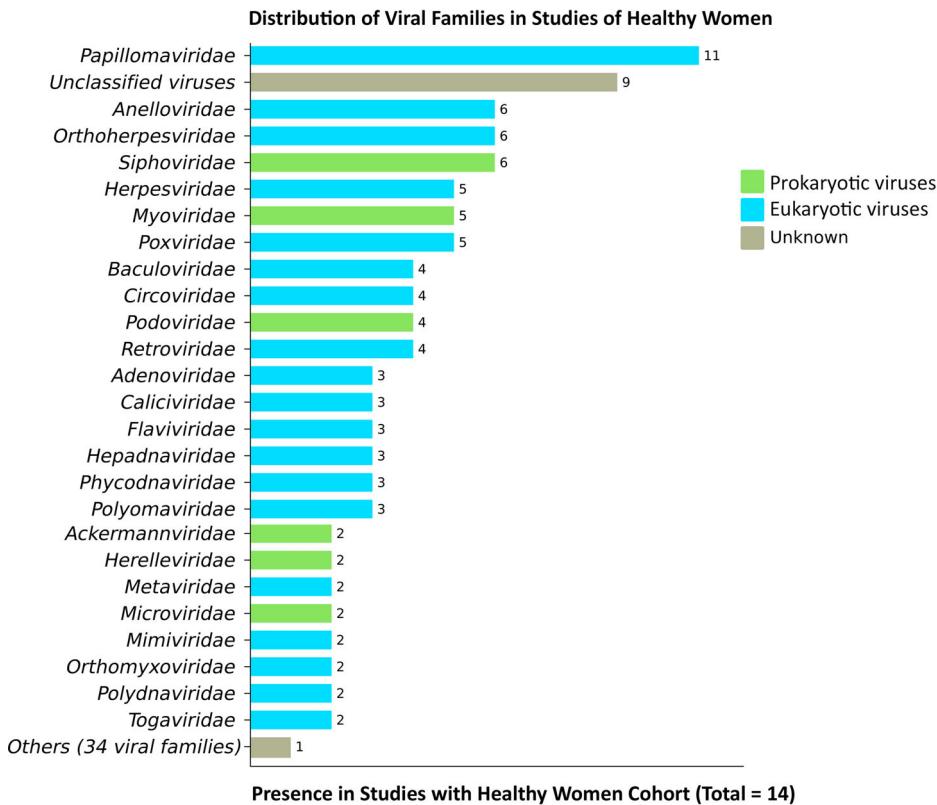
Fig. 3 | Distribution of viral families across different studies. Viral families are highlighted by host specificity, with prokaryotic viruses highlighted in green, eucaryotic viruses in blue, and unclassified viruses in orange.



constantly stimulate mucosal host responses, with potential consequences for host resistance to other infections and susceptibility to diseases such as asthma, type 1 diabetes and Crohn's disease, regarding respiratory and gastrointestinal tract virome studies⁴⁸. This raises the possibility that commensal viruses can behave differently under conditions of altered immunity, such as genital inflammation or HIV co-infection.

Furthermore, the presence of endogenous viruses, such as human endogenous retroviruses, highlights the complex role the eukaryotic virome may play in gene expression regulation, mutagenic events, and potentially gene transfer. These interactions, along with host genetic variations, might generate phenotypes that cannot be fully understood by studying the host or viruses in isolation¹¹.

Fig. 4 | Distribution of viral families reported in 14 studies with healthy women cohorts.



Finally, the presence of large genome viruses (e.g., *Poxviridae*, *Phycodnaviridae*, *Mimiviridae*) has been reported in several FRT virome studies, yet their role in human health and disease remains largely unexplored. While only members of *Poxviridae* and *Mimiviridae* families have been linked to disease in humans, the pathogenic potential of other Megavirales members, and their influence in the vaginal environment, requires further investigation^{43,49}.

Another area almost entirely lacking investigation is the characterization and the role played by viruses that can infect other microorganisms present in the vaginal environment. We lack information about the composition of the vaginal mycobiota and its relation with the virome, including whether viruses capable of infecting *Candida albicans*, are present in the FRT of some women and whether their occurrence influences the development or recurrence of vulvovaginal candidiasis. A virus that infects the protozoan *Trichomonas vaginalis*, has been identified, and its presence accentuates clinical disease in infected women⁵⁰.

Torque teno virus (TTV), a member of the *Anelloviridae* family, is typically regarded as a non-pathogenic endogenous virus commonly found in the majority of individuals. Its titer in biological fluids is known to vary depending on the immune status of the host⁵¹. Some studies have reported an increase in the abundance of *Anelloviridae* species in cases of genital inflammation due to HPV^{8,9,39}, but their role in health and disease remains largely unexplored. It would be of interest to determine whether TTV titer in the lower FRT could serve as a sensitive indicator of local immune status and a predictor of various pathological conditions, such as recurrent vulvovaginal candidiasis, cervical dysplasia, sexually transmitted infections, and pregnancy complications.

Infection of exocervical cells by pathogenic HPV types is a requisite early step in the development of cervical cancer⁵². In two studies investigating the FRT virome, HPV infection was shown to significantly influence the reduction of both viral and bacterial diversity, particularly in the context of inflammation^{17,33}. Genital inflammation, in turn, has been linked to an increased risk of cervical cancer progression and a heightened susceptibility

to acquiring sexually transmitted infections^{53,54}. Moreover, virome studies facilitated the identification of novel HPV types, which may contribute to tumorigenesis and other associated diseases^{13,40}. It remains to be determined whether the host immune system's ability to spontaneously clear an HPV infection is influenced by the co-occurrence of other viral and/or bacterial entities at the site. Conversely, the presence of another virus or bacterial species could potentially facilitate HPV persistence and increase infectivity. In an animal study it has been reported that the presence of a seemingly innocuous herpesvirus potentiated the pathogenic effect of a bacterial infection, leading to premature labor⁵⁵.

A study of DNA eukaryotic viruses in pregnant women has reported that while there was no association between the presence of a specific viral species and adverse outcome, there was an association between preterm birth and the presence of multiple viral species on the FRT, with changes in virome diversity mirroring those observed in the bacterial microbiome¹⁹. In contrast, another study that performed both DNA and RNA viral analysis observed that genital inflammation was associated with decreased virome richness and an increased abundance of *Anelloviridae* species in non-pregnant women⁸. A third study that also analyzed both DNA and RNA viruses at species level found greater viral diversity in healthy pregnant women compared to pregnant women with vaginitis³⁸. These associations between viral diversity and adverse consequences require further investigation.

Metagenomic studies are increasingly identifying new viruses, broadening our understanding of viral diversity and its roles in health and disease. Despite this progress, many viruses remain unclassified, and their functional roles are poorly understood. The taxonomic level of reporting is a critical factor that can influence the interpretation of results, as differences in reporting at the family, genus, or species level can lead to variations in how viral diversity and its association with health outcomes are understood. Characterizing these viruses, particularly those targeting vaginal pathogens, is crucial for exploring their potential in phage therapy. Comprehensive research is needed to fully characterize the FRT virome, as RNA virus detection remains challenging due to the instability of stored RNA and the

complexity of sequencing assays¹⁴. Additionally, the development of more robust viral databases and updated bioinformatics pipelines has improved the accuracy of virome analyses, highlighting the importance of public data sharing⁵⁶. There is also a lack of comparative analyses of virome composition among different ethnic groups to better understand potential virome variability influenced by genetic and environmental factors. Investigating the interactions between the virome, microbiome, and immune system is also essential for gaining deeper insights into their genomic contributions to women's health.

In conclusion, multiple species of eukaryotic and prokaryotic viruses reside in the lower FRT of healthy women. The specific viral entities present in any individual woman vary due to environmental and genetic variables. The role of specific viruses in maintaining reproductive tract health and the mechanisms leading to their contribution to pathology remains largely unexplored.

Methods

Literature search and selection criteria

To provide a comprehensive overview of studies employing viral metagenomic NGS in the female reproductive tract, a systematic review was conducted. Relevant studies were identified across five electronic databases: PubMed, Scopus, Embase, Web of Science, and Cochrane Library. Literature published up to April 14th, 2024, was included. Search terms were validated using the Health Sciences Descriptors (DeCS, WHO, 2024), with the search strategy utilized provided in the Supplementary Note 1. This study adhered to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA)⁵⁷ guidelines and was registered in the International Prospective Register of Systematic Reviews (PROSPERO; ID: CRD42021286295).

Study management and screening were facilitated by Rayyan web-based software. After duplicate removal, two reviewers screened titles and abstracts. Articles that passed this initial screening were subjected to a full-text review independently. No restrictions were placed on language or publication date during the selection process. Studies were included if they performed the characterization of the human FRT virome via metagenomic NGS. Exclusion criteria were: (1) absence of viral data (2) animal studies (3) studies in the form of conference abstracts, letters, or review articles; and (4) studies published solely as preprints without subsequent peer-reviewed publication.

Data extraction and analysis

For each included study, the following data were extracted: first author, journal, year of publication, study design (cross-sectional or longitudinal), cohort age, health conditions, sampling date and size, specimen type, geographical origin, *Lactobacillus* spp. dominance (where bacteriome analysis was conducted), and detected viral families. Viral taxonomy was cross-referenced using the NCBI Taxonomy Browser. Certain viral families reported in the original studies have undergone reclassification by the International Committee on Taxonomy of Viruses (ICTV). Specifically, the families *Siphoviridae*, *Myoviridae*, and *Podoviridae* were abolished in 2021, while *Herpesviridae* and *Totiviridae* were renamed to *Orthoherpesviridae* (2022) and *Orthotoviridae* (2023), respectively. Nevertheless, the original nomenclature used in each study was retained to preserve the systematic consistency of this review, alongside the most recent taxonomic context. Geographical data were stratified according to World Health Organization (WHO) regions: African Region, Americas Region, European Region, South-East Asia Region, and Western Pacific Region.

Technical and methodological metadata were also extracted, including enrichment and extraction method, type of nucleic acid extracted, sequencing platform utilized, application of internal controls, and data availability. Enrichment methods were categorized into five groups: Rolling Circle Amplification (RCA), targeted sequence capture, viral particle precipitation, methylation-based affinity enrichment, and filtration and centrifugation with RCA. Extraction methods were

categorized into four groups: silica-membrane based, solvent-based, magnetic beads based, and column-based. Sequencing platforms were grouped into three categories: Illumina, Ion Torrent, and BGI (Beijing Genomics Institute). Data availability was determined by whether the study deposited metagenomic data in public repositories such as ENA/SRA (European Nucleotide Archive and Sequence Read Archive), CNGBdb (China National GeneBank DataBase), or EBI (European Bioinformatics Institute).

Data availability

No datasets were generated or analysed during the current study.

Received: 25 August 2024; Accepted: 18 November 2024;

Published online: 25 November 2024

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Acknowledgements

This work was supported by the Virology laboratory (LIM-52) at the Instituto de Medicina Tropical, Faculdade de Medicina, Universidade de São Paulo.

Author contributions

L.H. conceptualized the project, conducted investigations, performed formal analysis, curated data and developed the methodology, performed validation and visualization, wrote the original draft, and reviewed and edited the manuscript. H.G.O.P. conducted investigations, formal analysis and validation, and reviewed the original draft. A.C.d.C. performed formal analysis and reviewed the manuscript. T.R.T.-M. performed formal analysis, reviewed the original draft and the manuscript. M.C.M.-C. supervised the project, reviewed the original draft and the manuscript, and secured funding. S.S.W. supervised the project, performed formal analysis, wrote the original draft, and reviewed and edited the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

Supplementary information The online version contains supplementary material available at <https://doi.org/10.1038/s41522-024-00613-6>.

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