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Fecal virome at the human-animal interface: a one health perspective on an uncharted frontier

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Abstract
The exponential growth of the human population and associated intensifications in animal farming, pet ownership, and habitat anthropisation have dramatically increased human-animal interactions. Global livestock production now exceeds 24 billion animals annually, and pet ownership has risen to over 70% of households in many developed nations, creating unprecedented interfaces for viral exchange. This heightened contact has multiplied opportunities for zoonotic and reverse-zoonotic transmission, as tragically exemplified by the SARS-CoV-2 pandemic. The fecal virome—defined as the totality of viral nucleic acids in the gastrointestinal tract—represents a crucial, yet largely unexplored, pathway for such exchanges. While the bacterial microbiome's role is increasingly recognized, the virome's composition, dynamics, and transmissibility between co-habiting humans and animals remain poorly characterized. This review compiles current evidence on the fecal virome of key domestic animals (equines, livestock, pets) and their human contacts under the "One Health" framework. We critically evaluate methodological approaches—from targeted PCR to viral metagenomics—and highlight the discovery of novel viruses and identification of zoonotic agents through metagenomic approaches. Critically, we identify significant knowledge gaps, including the absence of definitive evidence for contemporary cross-species transmission versus shared ancestry or convergent evolution. We propose a strategic research agenda focused on longitudinal studies of human-animal cohorts, standardized metagenomic methodologies, and functional analyses of the virome. Elucidating the fecal virome at this interface is paramount for developing proactive surveillance strategies to predict and prevent the next emerging viral disease.

Keywords Fecal virome, Animals, Humans, Zoonoses, One-health, Viral metagenomics

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Introduction: the one health imperative and the viromic blind spot

The increasing frequency of emerging infectious diseases of animal origin (zoonoses) underscores the inextricable linkages between human, animal, and environmental health [1, 2]. The "One Health" concept provides an essential framework for understanding these connections [3]. Drivers such as intensive livestock farming, expanding pet ownership, and ecosystem encroachment have created an unprecedented level of intimacy at the human-animal interface [4, 5].

Global trends in human-animal contact: Contrary to assumptions that urbanization reduces human-animal interactions, evidence demonstrates **increased contact rates in specific contexts**. Global livestock production has intensified dramatically, with the number of farmed animals increasing from approximately 7.3 billion in 1970 to over 24 billion in 2020 [FAOSTAT, 2021]. Peri-urban livestock farming now supplies much of the animal protein to cities, creating dense human-animal interfaces at the urban fringe [6]. Live animal markets in urban centers concentrate multiple species in high-traffic settings, facilitating viral exchange [7]. Concurrently, global pet ownership has demonstrably increased—for example, in the United States, the percentage of households owning pets rose from 56% in 1988 to 70% in 2020, with similar trends reported in Europe and Asia [American Pet Products Association, 2021; European Pet Food Industry, 2022]. These trends collectively argue for intensified, not diminished, human-animal viral exchange potential.

While the bacterial microbiome within this interface has been extensively studied, the viral component—the virome—has lagged [8]. The fecal virome, comprising the genetic content of all viruses inhabiting the gastrointestinal tract, is a particularly rich source of viral diversity and a potential hotspot for cross-species transmission [9]. This review argues that the systematic exploration of the fecal virome is a critical new frontier in One Health, with the potential to revolutionize our understanding of viral zoonoses, inform diagnostic tool development, and enhance pandemic preparedness.

Viruses with pandemic potential utilizing fecal-oral transmission: While respiratory viruses dominate current pandemic discourse, several viral families with fecal-oral transmission routes possess significant emergence potential: (1) **Hepatitis E virus** (*Hepeviridae*), particularly genotype 3, circulates asymptomatically in swine and has caused increasing human cases through foodborne and environmental transmission [10]; (2) **Enteroviruses**, including *Enterovirus* D68 and D70, have demonstrated capacity for neurotropic evolution and global spread despite primarily fecal-oral transmission [11]; (3) **Astroviruses** and **Sapoviruses**, long considered benign, are increasingly associated with extra-intestinal disease and show evidence of cross-species transmission [12]; (4) **Rotaviruses**, while vaccine-preventable in humans, continue to evolve in animal reservoirs with potential for reassortment [13]; and (5) **Coronaviruses**, while primarily respiratory, exhibit enteric replication and fecal shedding (as documented for SARS-CoV-2, MERS-CoV, and multiple animal coronaviruses), suggesting the gastrointestinal tract may serve as a cryptic reservoir and evolutionary crucible [14]. A more thorough understanding of the viral diversity present in natural environments, coupled with a comprehensive grasp of potential host species and their ecological interactions, is crucial for predicting and mitigating future zoonotic spillover events [15]. This comprehensive approach necessitates advanced metagenomic techniques to characterize both known and novel viral agents across diverse animal populations and environmental niches [16] Fig 1.

The increasing global population further intensifies these interactions, amplifying the risk of zoonotic pathogen transmission from wildlife, livestock, and domestic animals to humans [17]. This close proximity facilitates the interspecies transmission of an estimated **75% of all emerging virus-associated infectious diseases**, as demonstrated by recent outbreaks like Zika, Ebola, and COVID-19 [17]. The World Organization for Animal Health (WOAH) has recently stated that public health crises resulting from viruses of potential animal origin highlight the need for the One Health approach in understanding and confronting global health risks. Furthermore, WOAH advises that close contact with infected animals can represent a potential source of infection in humans, as demonstrated for SARS-CoV-2 [18].

This review synthesizes our current understanding of the fecal virome in key domestic animals, critically evaluates the evidence for viral exchange at the human-animal interface, and outlines a strategic research agenda to harness this knowledge for improving global health security.

The fecal virome: concepts, methods, and significance

The virome, encompassing the entire community of viruses in a specific niche, includes both eukaryotic viruses that infect host cells and bacteriophages (phages) that infect prokaryotes. The fecal virome represents a particularly dense and dynamic viral ecosystem. Its significance is twofold. First, eukaryotic viruses can be direct pathogens, causing diseases ranging from acute gastroenteritis to systemic infections. Second, and perhaps more pervasively, bacteriophages exert a profound indirect influence on host health. Through predator-prey dynamics (the "kill-the-winner" model) and horizontal gene transfer (transduction), phages are potent drivers of bacterial community structure and function [19, 20].

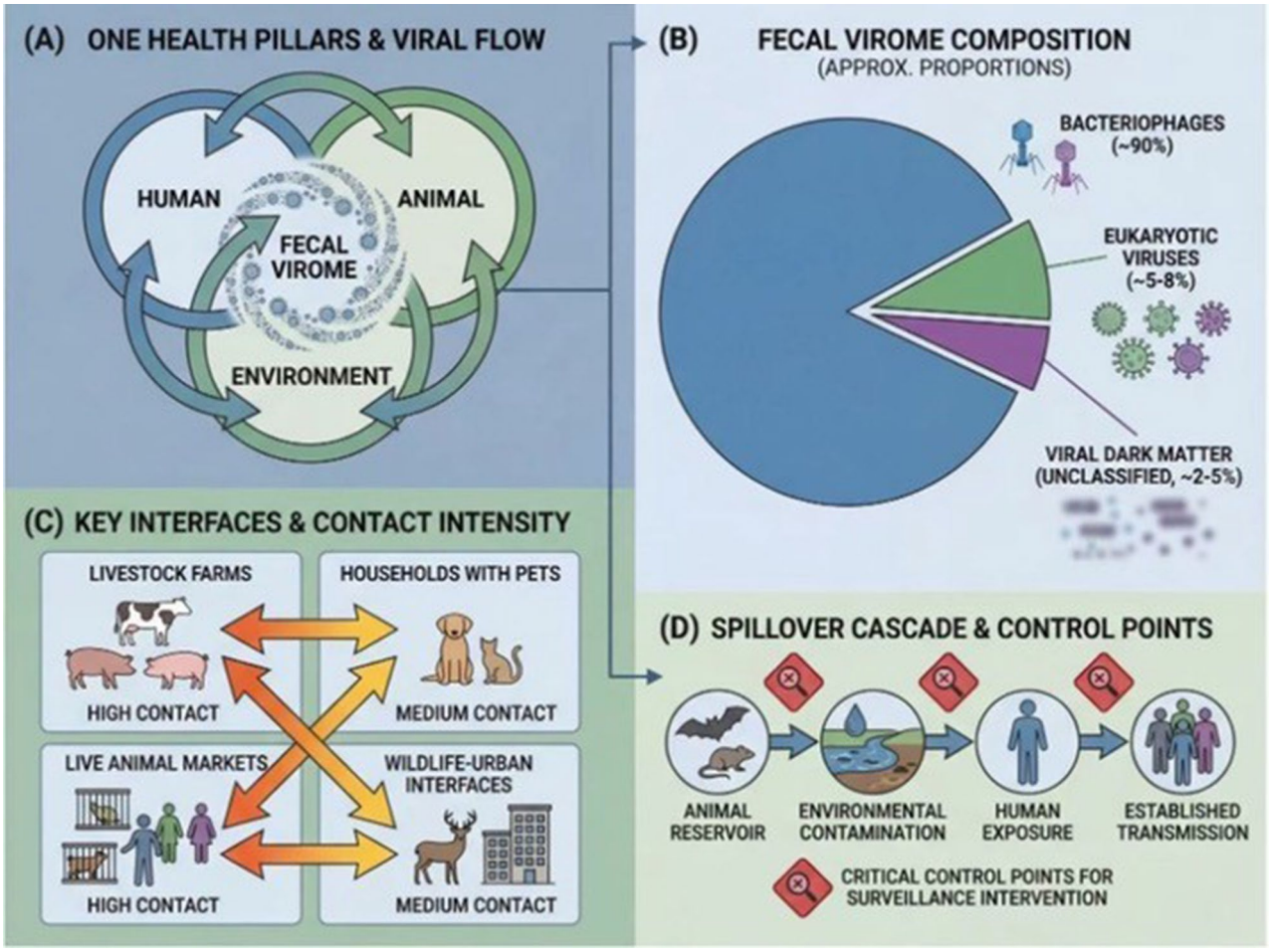

Fig. 1 Conceptual framework illustrating the fecal virome at the human-animal interface. (**A**) The three pillars of One Health (human, animal, environment) with bidirectional viral flow arrows. (**B**) Composition of the fecal virome showing approximate proportions of bacteriophages (~90%), eukaryotic viruses (~5-8%), and viral dark matter (unclassified sequences, ~2-5%) based on current literature. (**C**) Key interfaces with relative contact intensity: livestock farms, households with pets, live animal markets, wildlife-urban interfaces. (**D**) The spillover cascade from animal reservoir → environmental contamination → human exposure → established transmission, with critical control points for surveillance intervention

Phage-mediated virulence: a classic example: The paradigmatic example of phage-mediated horizontal gene transfer is *Vibrio cholerae*, where the genes encoding cholera toxin (*ctxAB*) are carried by the filamentous phage CTXϕ. Lysogenic conversion of environmental or human-associated *V. cholerae* strains by CTXϕ directly determines pandemic potential, demonstrating how bacteriophages can fundamentally shape disease emergence and severity [21]. Similarly, phage-encoded Shiga toxins in enterohemorrhagic *E. coli* (EHEC) and various phage-mediated antibiotic resistance genes illustrate the profound, though often invisible, role of the virome in shaping bacterial pathogenicity at the human-animal interface [22].

By lysing dominant bacterial strains or shuttling genes for functions like nutrient metabolism or antibiotic resistance, phages can fundamentally alter the gut microbiome, thereby influencing states of health and disease. For instance, **inflammatory bowel disease** (IBD) has been associated with expansion of *Caudovirales* bacteriophages in Crohn's disease patients [23, 24]; **obesity** shows altered virome composition with reduced viral richness and specific phage signatures [25]; and **type 1 diabetes** exhibits enterovirus signatures in pancreatic tissue and altered enteric virome composition [26].

Beyond chronic conditions, the virome's influence on infectious disease susceptibility is increasingly recognized. *Murine Norovirus*, a common constituent of the mouse enteric virome, can substitute for bacterial signals in restoring intestinal morphology and lymphocyte function, thereby modulating susceptibility to bacterial and parasitic infections [27]. In humans, baseline enteric virome composition has been associated with differential outcomes in enteric pathogen infections and vaccine responses [28], suggesting that the virome itself may constitute a determinant of infectious disease susceptibility.

Table 1 Comparison of methods for viral detection across the detection-characterization-surveillance continuum, including strengths, limitations, and applications

Method	Target	Strengths	Limitations	Application Stage
Targeted PCR/qPCR/dPCR	Specific known viruses	High sensitivity (detection limits 10–100 copies/mL); quantitative; low cost per sample; rapid turnaround	Requires prior sequence knowledge; narrow scope; cannot detect novel viruses	Detection (known threats)
Amplicon sequencing	Conserved regions (polymerase, capsid, hemagglutinin, spike)	Broader detection within viral families; captures variants; lower cost than metagenomics; enables phylogenetic analysis	PCR bias; requires conserved primer design (may miss divergent viruses); limited to targeted families	Detection/ Characterization
Viral metagenomics (mNGS)	All viral nucleic acids	Unbiased discovery potential; novel virus detection; full genome recovery possible; captures entire viral community	High cost ($500–1000/sample); computationally intensive; database-dependent; low-abundance virus detection challenges; requires viral enrichment	Discovery/ Characterization
Hybrid capture enrichment	Viral genomes (targeted or broad panels)	Increased sensitivity for specific viruses (100–1000× enrichment); reduces host background; cost-effective for large studies	Requires probe design; may miss unexpected viruses; initial setup cost high	Characterization/ Surveillance
Serological assays	Host antibody response	Indicates past exposure; can survey large populations; inexpensive	Cannot detect active shedding; cross-reactivity issues; requires species-specific reagents	Surveillance
Wastewater-based epidemiology	Community-level viral pools	Population-level surveillance (thousands to millions covered); non-invasive; early outbreak detection potential	Cannot link to specific individuals; dilution effects; environmental degradation of nucleic acids	Surveillance

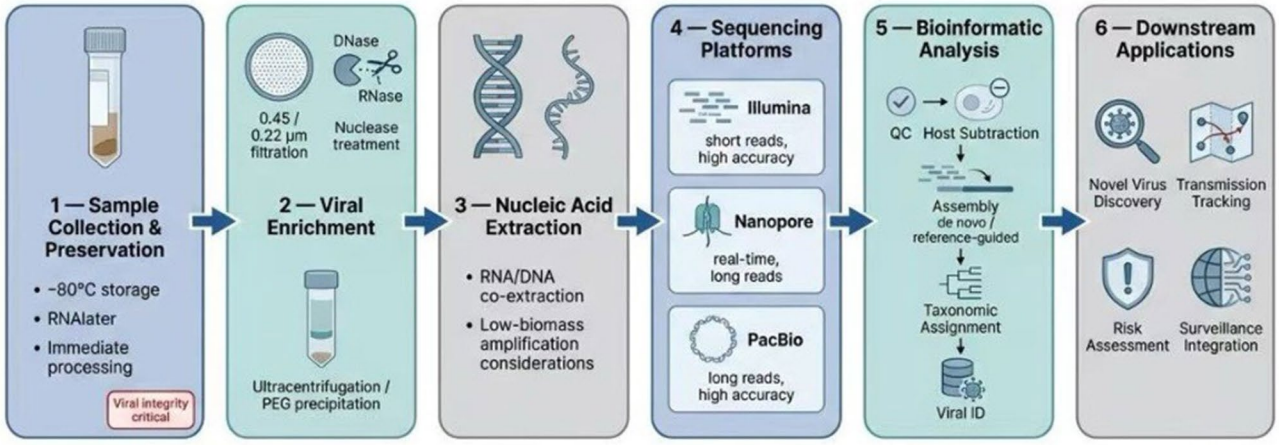

Fig. 2 Workflow for fecal virome analysis from sample to interpretation. (1) Sample collection and preservation (viral integrity critical; options: −80°C, RNAlater, or immediate processing). (2) Viral enrichment strategies: filtration (0.45 µm/0.22 µm removes bacteria and eukaryotes), nuclease treatment (digests free non-encapsidated nucleic acids), ultracentrifugation or PEG precipitation (concentrates viral particles). (3) Nucleic acid extraction: considerations for RNA/DNA co-extraction, low-biomass amplification. (4) Sequencing platforms: **Illumina** (high accuracy, short reads), **Nanopore** (real-time, long reads), **PacBio** (long reads, high accuracy). (5) Bioinformatic analysis: quality control, host subtraction, assembly (de novo or reference-guided), taxonomic assignment, viral identification. (6) Downstream applications: novel virus discovery, transmission tracking, risk assessment, surveillance integration. Adapted from [34] and [35]

Methodological approaches to fecal virome analysis: a critical comparison

The advent of high-throughput metagenomic next-generation sequencing (mNGS) has been transformative for virome exploration, enabling **broader**, **less targeted** discovery compared to culture-dependent or PCR-based methods [29]. However, it is critical to acknowledge that mNGS is not truly "unbiased." Every step—from sample collection and storage, to viral enrichment, nucleic acid extraction, library preparation, and bioinformatic analysis—introduces distinct biases [30, 31] Table 1.

Amplicon-based approaches deserve particular consideration for their utility in targeted surveillance. Unlike metagenomics, which sequences all nucleic acids, amplicon methods use conserved primers to amplify

specific genomic regions—such as the **hemagglutinin gene of influenza viruses**, the **spike protein gene of coronaviruses**, or the **capsid protein genes of enteric viruses**—enabling sensitive detection and variant characterization within viral families of interest [32]. These methods bridge the gap between targeted PCR (narrow but sensitive) and metagenomics (broad but less sensitive), offering a cost-effective means to surveil viral families with pandemic potential. However, limitations include primer-template mismatches in divergent strains and the inability to detect truly novel viruses outside the targeted families [33]. The choice among these methods depends on the research question: discovery-oriented studies require metagenomics, while surveillance of known threat families may be better served by amplicon or hybrid capture approaches.

Bioinformatics challenges and tools for virome analysis

Bioinformatic analysis of virome data presents distinct challenges that vary between bacteriophages and eukaryotic viruses [36]:

For bacteriophages:

- *Advantages*: High abundance in fecal samples (typically 90%+ of viral reads); well-developed phage-specific databases (e.g., IMG/VR, PhagesDB); tools optimized for phage discovery (VirSorter2, VIBRANT, PhaBOX)
- *Limitations*: Extreme genetic diversity complicates taxonomic assignment; high proportion of hypothetical proteins (often >70%); difficulty linking phages to bacterial hosts

For eukaryotic viruses:

- *Advantages*: Better representation in reference databases (RefSeq, GenBank); conserved genes (polymerase, capsid) facilitate annotation
- *Limitations*: Low abundance relative to phages and host DNA (often <1% of sequencing reads); requires extensive host subtraction; **lack of universal marker genes** (equivalent to 16S rRNA) complicates diversity estimation; reference databases heavily biased toward pathogenic viruses of humans and livestock, underrepresenting commensal and wildlife viruses [37] Table 2

Key bioinformatic limitations requiring further development

1. **The 'viral dark matter' problem**: 50–90% of viral reads have no match in reference databases [40], limiting taxonomic resolution and leaving vast diversity uncharacterized.

2. **Assembly challenges**: Viral genomes often contain repeats, exist as quasispecies, and have variable coverage, complicating *de novo* assembly from short reads. Recent benchmarking shows that **combining multiple**

Table 2 Common bioinformatic tools for virome analysis, their functions, strengths, and limitations. Compiled from recent benchmarking studies [36, 38, 39]

Tool	Function	Strengths	Limitations
Kraken2/Bracken	Taxonomic classification	Fast, user-friendly; memory-efficient with MiniKraken databases	Database-dependent; misclassification of novel viruses; limited to known sequences
Diamond/MEGAN	Alignment-based annotation	Sensitive for divergent sequences; handles large datasets	Computationally intensive; requires significant memory
VirSorter2/VIBRANT	Phage identification	Machine learning-based; detects diverse phages including novel lineages	Primarily optimized for phages; eukaryotic virus detection limited
VirFinder	Phage identification	k-mer based, alignment-free; fast	Higher false positive rate; requires length filtering
CheckV	Genome completeness assessment	Quality control for viral genomes; estimates completeness and contamination	Requires assembled contigs; database-dependent for host contamination
ViralVerify	Eukaryotic virus confirmation	Specifically designed for eukaryotic viruses; low false positives	Limited to known viral families; lower sensitivity for highly divergent viruses
VIP (Virus Identification Pipeline)	Integrated viral detection	Combines multiple tools; comprehensive reporting	Complex installation; resource-intensive
FastViromeExplorer	Read-based profiling	High sensitivity; detects low-abundance viruses; good for eukaryotic viruses	High false positive rate with closely related strains [39]
ViromeScan	Stringent viral detection	Conservative; low false positives; good for clinical applications	Low sensitivity; misses many viruses [39]
metaFlye/MEGAHIT	Assembly (long/short reads)	Optimized for viral genomes; handles repeats well	Parameter tuning required; computationally intensive
CONCOCT/MetaBAT2	Viral binning	Clusters viral contigs into populations	Performance varies by data type; CONCOCT may cluster unrelated contigs [38]

assemblers increases high-quality viral genome recovery by 4.8- to 21.7-fold compared to single assemblers [38].

3. **Strain-level resolution**: Standard pipelines often collapse closely related strains, obscuring fine-scale transmission dynamics critical for outbreak investigation.

4. **Platform-dependent biases**: Read-based profilers yield divergent virome portraits driven by database scope and mapping stringency. FastViromeExplorer detects the most taxa overall, while ViromeScan preferentially reports eukaryotic and human viruses [39].

5. **Lack of gold standards**: No universally accepted benchmarks for comparing bioinformatic tools; database versions and parameters are inconsistently reported, hampering cross-study comparability [41].

Emerging solutions include long-read sequencing (Oxford Nanopore, PacBio) for improved assembly [38], machine learning approaches for novel virus detection [42], and community efforts to expand reference databases (e.g., the Global Virome Project, the Human Virome Database).

In healthy human adults, the fecal virome exhibits remarkable temporal stability over months to years, yet striking inter-individual variability [43, 44]. A longitudinal study of healthy adults found that while within-individual viral composition remained stable (mean similarity index > 0.8 over 1 year), between-individual similarity was < 0.3, with each individual harboring **5–25 distinct dominant viral genotypes** [45]. In healthy livestock, similar patterns of high inter-individual variability have been observed, though longitudinal data are sparser. In swine, viral richness estimates range from **15 to 50 viral genotypes per individual**, with *Microviridae* and *Caudoviricetes* phages predominating, but with marked variation across age groups, farms, and individuals [46, 47].

The gastrointestinal tract serves as a reservoir for genetic exchange—particularly through phage-mediated transduction and eukaryotic virus co-infection enabling recombination—creating conditions conducive to viral evolution. The high density of microbial hosts, constant immune pressure, and potential for co-infection with diverse viral lineages make the gastrointestinal tract a particularly dynamic arena for viral genetic diversification and potential emergence of novel variants [48].

The animal fecal virome: a reservoir of known and unknown threats

Metagenomic studies have begun to map the immense viral diversity within the animal gut, consistently revealing two key themes: the discovery of novel viral lineages and the identification of animal homologs and reservoirs of known human pathogens.

Livestock

Studies in cattle and pigs have identified homologs of human hepatitis viruses (e.g., **Hepaciviruses**, **Pegiviruses**), suggesting a broader host range and providing potential new models for studying human viral pathogenesis [29, 30]. A recent metagenomic survey of swine fecal samples from 10 farms identified **over 700 viral genotypes**, including 47 previously undescribed genera across 12 viral families [49]. In cattle, viral richness estimates range from **30 to 120 viral genotypes per sample**, with cumulative diversity across herds exceeding 1,500 viral genotypes [50]. This diversity, while immense, remains poorly characterized relative to bacterial microbiomes and likely represents a substantial underestimation due to database limitations and methodological biases.

Comparative studies have shown distinct virome profiles between cattle and horses on the same farm [51]. While this finding is consistent with host-specific factors shaping viral communities—such as differences in gastrointestinal physiology, immune systems, diet, and bacterial microbiota—it is critical to note that correlation does not establish causation. The observed differences could equally reflect host-specific bacterial communities (which shape the phage population), different exposure histories, or stochastic founder effects. Importantly, while the cattle and equine viromes are not "interacting" directly, they may indirectly influence each other through shared environmental viral pools (e.g., contaminated water, feed, or fomites), creating opportunities for spillover that would not be evident from cross-sectional comparisons alone.

Equines

Horses, due to their close historical association with humans, are a key species of interest. Research has identified novel parvoviruses and has extensively characterized *Equine Coronavirus* (ECoV), which shares clinical and molecular features with other zoonotic coronaviruses [52, 53]. Furthermore, horses have been identified as hosts for diverse enteric viruses with zoonotic potential or phylogenetic relatedness to human pathogens, including **hepatitis E virus (HEV) genotype 3** [54], novel parvoviruses [55], and coronaviruses that exhibit fecal shedding [56]. This intersection highlights the necessity of sustained surveillance and research into equine enteric viromes to proactively mitigate potential zoonotic threats, aligning with the broader objectives of global health security.

Companion animals

The fecal virome of dogs and cats is increasingly recognized as diverse, including families like *Astroviridae*, *Caliciviridae*, *Coronaviridae*, and *Parvoviridae* [57, 58]. Outbreaks of vomiting and diarrhea in **animal shelters** (particularly in dogs and cats) have been linked to novel

viruses such as astroviruses and bocaviruses [59, 60]. While these outbreaks have primarily been documented in animal populations, the close phylogenetic relationships of some of these viruses to human astroviruses and bocaviruses, combined with the intense human-animal contact in shelter and home environments, raises questions about their **potential** for zoonotic transmission and their broader pathogenic significance [61]. Prospective studies have yet to definitively demonstrate cross-species transmission of these agents, highlighting a critical knowledge gap.

Wild animals

Wild animal populations represent vast, largely unexplored reservoirs of viral diversity, with metagenomic studies continually uncovering novel viruses that challenge existing phylogenetic classifications and expand our understanding of viral evolution [62]. Studies of wildlife viromes, particularly in species such as bats, have identified novel viral lineages and underscored their potential relevance to both human and livestock health, necessitating enhanced infectious disease monitoring [63]. Recent bat virome studies employing longitudinal sampling approaches (e.g. [64]), have demonstrated that individual bats harbor dynamic viral communities with substantial turnover over time, emphasizing the need for repeated sampling to capture true viral diversity.

The Global Virome Project aims to discover over **70% of currently unknown viral diversity** in mammals and water birds, underscoring the critical need for comprehensive viral surveillance in these populations [65]. The emergence of infectious diseases like COVID-19 underscores the critical importance of understanding these interspecies connections for disease control and prevention [66].

Documented examples of cross-species fecal-oral transmission While much of the evidence for viral sharing remains circumstantial, documented cases do exist and provide proof-of-concept for the pathways we hypothesize.

- ***Rotavirus*** **G and P genotype sharing** between humans and livestock has been repeatedly documented, with evidence of both zoonotic and anthroponotic transmission [67, 68]
- **Hepatitis E virus genotype 3** circulates between swine and humans with demonstrated foodborne and environmental transmission [69]
- **Porcine sapoviruses** have been detected in human stool samples with high sequence identity to swine strains [70]
- **Noroviruses** exhibit evidence of zoonotic transmission, with GII.P18/GII.18 strains detected in both swine and humans [71]

These cases demonstrate that cross-species fecal-oral transmission occurs, providing an evidentiary foundation for broader surveillance efforts.

The human-animal interface: a hypothesis-generating arena

The central, and largely untested, hypothesis in this field is that individuals with close animal contact (farmers, veterinarians, pet owners) harbor fecal viromes that are more similar to their animals than the general population. Preliminary evidence is suggestive: the finding of HCV-related viruses in dogs and horses, and shared virome features between co-habiting species, points to possible cross-species transmission or shared environmental exposures [29, 30]. However, current data are fragmented. Most studies focus on a single species during disease outbreaks, lacking the parallel, longitudinal sampling of humans and their contact animals required to demonstrate transmission and establish directionality.

We must emphasize a crucial caveat: the evidence presented thus far documents phylogenetic relatedness and ecological association, not demonstrated cross-species transmission. The discovery of HCV homologs in horses and dogs, for instance, while suggestive of common ancestry or past cross-species events, could equally reflect long-term co-divergence with host lineages or convergent evolution in the absence of contemporary transmission [72]. Distinguishing these possibilities requires experimental evidence (e.g., receptor compatibility studies, infection models) and longitudinal epidemiological data that currently do not exist for most animal viruses Fig 3.

This knowledge gap highlights a critical need for integrated, longitudinal studies that concurrently analyze human and animal viromes within shared environments to precisely delineate host-specific factors and zoonotic transmission events [73]. Such research is pivotal for understanding viral ecology, predicting zoonotic spillover risks, and developing targeted interventions to mitigate disease transmission at the human-animal interface [74].

This leads to our first testable hypothesis: **Individuals with occupational or residential close contact with animals (farmers, veterinarians, slaughterhouse workers, pet owners) will harbor enteric viruses with higher sequence identity to those found in their contact animals compared to matched controls without such contact**. Testing this hypothesis requires carefully designed case-control studies with parallel sampling of humans and animals, standardized virome methods, and statistical approaches to account for shared

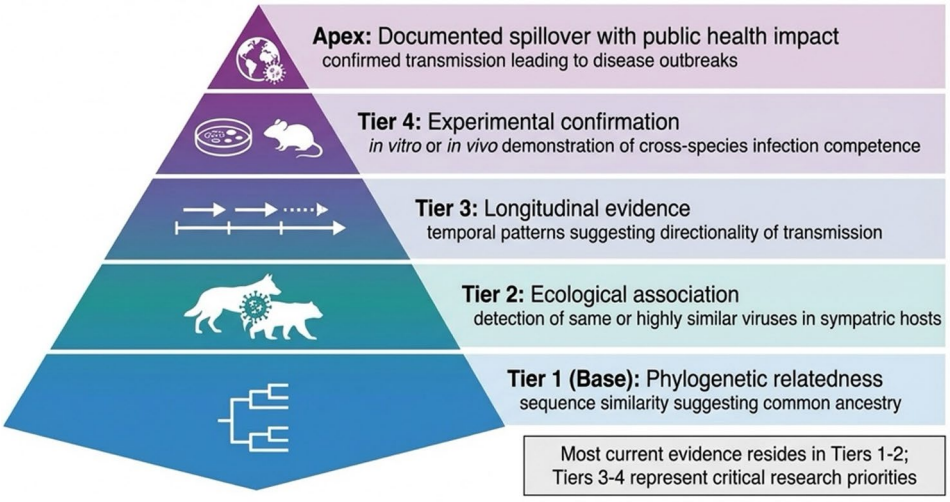

Fig. 3 Hierarchical framework for evaluating evidence of viral sharing across species. Tier 1 (Base): Phylogenetic relatedness—sequence similarity suggesting common ancestry. Tier 2: Ecological association—detection of same or highly similar viruses in sympatric hosts. Tier 3: Longitudinal evidence—temporal patterns suggesting directionality of transmission. Tier 4: Experimental confirmation—in vitro or in vivo demonstration of cross-species infection competence. Apex: Documented spillover with public health impact—confirmed transmission leading to disease outbreaks. Most current evidence resides in Tiers 1–2; Tiers 3–4 represent critical research priorities

environmental exposures versus true transmission. Such studies could provide the first direct evidence for the magnitude and directionality of viral exchange at the human-animal interface.

Recent research has identified a significant overlap in *Circovirus* and *Parvovirus* strains circulating between wildlife and domestic animals, complicating disease control efforts across species [75]. This interplay between wildlife, domestic animals, and humans underscores the complex dynamics of viral transmission and the urgent need for comprehensive studies that encompass all three domains [75]. For instance, approximately **89% of recognized RNA viruses with zoonotic potential** emphasize the need for surveillance migration to tropical hotspots in Africa, Latin America, and Asia, where human expansion into new ecosystems and the wildlife trade increase pathogen transmission risk [76].

Furthermore, anthropogenic land-use changes significantly influence host viromes by **altering host species composition** (which animals are present), **host population densities** (how many individuals and how closely they interact), and **contact patterns** between species. These ecological perturbations can reshape viral communities and increase contact rates between previously separated species, thereby elevating spillover risk [77, 78].

Future perspectives and research agenda

To move from correlation to causation, a coordinated One Health research agenda is essential. Key priorities include:

Longitudinal cohort studies

Establishing well-defined cohorts of human-animal pairs with repeated sampling to track virome dynamics and viral exchange over time. Such studies are critical for discerning temporal patterns of viral shedding, persistence, and potential transmission events within shared environments, thereby elucidating factors that drive viral adaptation and host shifts [79, 80].

An ideal flagship project would be A multi-year study of **100 farmer-pig pairs** and **100 dog-owner pairs**, with monthly fecal sampling and detailed metadata collection on health, diet, and behavior.

- **Why pigs?** Pigs are well-documented reservoirs for viruses with zoonotic potential (hepatitis E virus, influenza A viruses, porcine coronaviruses, rotaviruses), have high human contact intensity in farming systems, and possess gastrointestinal physiology sufficiently similar to humans to warrant concern about shared viral susceptibility [81, 82]
- **Why dogs?** Dogs share the domestic environment intimately with humans, have high contact rates (including fecal-oral exposure through licking, shared surfaces, and indoor co-habitation), and mounting evidence suggests they harbor diverse enteric viruses related to human pathogens [83, 84]
- **Why 100 pairs?** Power calculations based on expected viral diversity (estimated 20-50 viral genotypes per individual) and anticipated effect sizes from pilot studies suggest that 100 pairs would provide **80% power to detect a 20% difference**

in viral sharing between exposed and unexposed groups, accounting for clustering and dropout. This sample size also enables subgroup analyses by farming system, biosecurity practices, and animal age
- **Why monthly sampling?** Viral shedding is episodic. Monthly sampling over 2-3 years captures seasonal variation, intermittent shedding, and enables reconstruction of transmission networks using viral evolutionary rates (molecular clock analysis) [85]

Methodological standardization

Developing consensus protocols for sample processing, viral enrichment, and bioinformatic analysis to enable meaningful cross-study comparisons. This harmonization of techniques is crucial for generating reproducible and comparable data across diverse research groups, thereby accelerating our collective understanding of viral ecology [86].

Critical standardization needs include

- **Minimum information standards** for virome studies (analogous to MIxS for metagenomics)
- **Reference materials and spike-in controls** for cross-laboratory calibration
- **Shared benchmark datasets** for tool validation and comparison
- **Open-source bioinformatic workflows** with version-controlled databases
- **Reporting guidelines** for database versions, parameters, and quality metrics

Furthermore, the application of unbiased metagenomic next-generation sequencing across various animal taxa will illuminate viral evolution and host responses, providing a more comprehensive view of the animal virome [76]. Continued innovation in these areas will facilitate the discovery of novel viruses and the elucidation of complex virus-host interactions, which are critical for predicting zoonotic spillover events [17].

Functional studies

Moving beyond sequencing to investigate the biological activity and host response to newly identified viruses, using in vitro and in vivo models. Such studies are essential for understanding viral pathogenesis, tropism, and the mechanisms underpinning interspecies transmission, bridging the gap between genomic detection and functional characterization of emerging pathogens [87].

Priority functional investigations

- **Receptor compatibility studies** using pseudotyped viruses and cell lines from different species
- **Animal model infections** to assess tropism, shedding, and pathogenicity
- **Organoid cultures** from human and animal intestinal epithelium for comparative infection studies
- **Serological surveys** to detect past exposure in high-risk human populations

These functional investigations are vital for assessing the zoonotic potential of newly discovered viruses and for developing targeted antiviral strategies and vaccines [88]. A deeper understanding of existing viral populations and their ecological characteristics is imperative for detecting potential interspecies spillover events, particularly given the widespread application of molecular techniques in virus identification and functional analyses [89].

Diagnostic translation

Leveraging virome data to develop broad-spectrum or pan-viral diagnostic assays for surveillance and early detection of emerging threats. This approach is crucial for translating research findings into actionable public health tools, allowing for rapid identification and characterization of novel viral agents before they cause widespread outbreaks [90].

Priority diagnostic developments

- **Pan-viral microarray platforms** targeting conserved regions across viral families
- **Multiplex PCR panels** for high-risk viral families identified through virome surveillance
- **CRISPR-based detection systems** for rapid, field-deployable identification
- **Wastewater surveillance protocols** optimized for emerging zoonotic viruses

Novel diagnostic platforms, such as the GeneXpert™, offer rapid, multiplexed detection of a broad spectrum of human pathogens and are continuously being refined to enhance sensitivity and expand pathogen coverage [91]. These advancements in diagnostic capabilities, coupled with enhanced surveillance, will enable timely identification of emerging viral threats and facilitate rapid responses to prevent potential pandemics [92].

This research agenda must be globally inclusive. Socioeconomic factors, such as poverty and limited veterinary infrastructure, exacerbate zoonotic risk [93, 94]. Therefore, longitudinal studies and surveillance efforts must be deliberately implemented in low-resource settings, where

human-animal interfaces are often most intense. This requires coupling basic virome research with initiatives to strengthen local veterinary and public health capacity, ensuring that the benefits of One Health are equitably distributed.

Addressing these vulnerabilities requires a concerted global effort, integrating social sciences to promote behavioral modifications and public awareness regarding high-risk human-animal interactions, such as wildlife trade and bushmeat consumption [95]. Furthermore, interdisciplinary collaborations between epidemiologists, ecologists, veterinarians, and social scientists are critical for developing comprehensive and sustainable strategies to mitigate the risks associated with zoonotic diseases and predict their emergence [96].

Discussion

Given the increasingly interconnected nature of human, animal, and environmental health, the One Health approach has become critically important in understanding and addressing complex health challenges like zoonotic diseases [97]. This holistic framework recognizes that the health of humans is intrinsically linked to the health of animals and the environment they inhabit [98]. This integrated perspective is essential for developing comprehensive strategies to mitigate risks associated with emerging infectious diseases and food insecurity, ultimately contributing to the Sustainable Development Goals [99].

The One Health paradigm emphasizes proactive surveillance of pathogens in wildlife, particularly given that most zoonotic pathogens originate from animal populations, thereby enabling early detection and intervention strategies [100]. This integrated approach is particularly vital in mitigating the impact of fecal contamination, where viral metagenomics can effectively track sources and assess risk to inform targeted interventions for both human and animal populations [16].

Wastewater surveillance: capabilities and limitations: The application of viral metagenomics to wastewater surveillance provides a centralized, cost-effective method for monitoring viral spread within communities, as demonstrated during the SARS-CoV-2 pandemic [17]. However, it is crucial to note that these systems detect pathogens **already circulating** in human populations; they did not provide early warning of the emergence event itself. The COVID-19 pandemic thus serves as a powerful example of the **gap** in pre-emergence surveillance: by the time SARS-CoV-2 was detected in wastewater or clinical cases, widespread human transmission was already underway [101]. This underscores the need for surveillance systems that operate **before spillover**, at the animal-human interface, to complement downstream monitoring of established human transmission.

The One Welfare concept expands upon One Health by integrating animal well-being and broader ecological and social contexts, acknowledging their deep interconnectedness [102]. This comprehensive view necessitates collaborative efforts across diverse disciplines, including human health, animal health, and environmental sciences, to create a unified detection and response infrastructure [84, 100]. A formalized national and international network of laboratories operating under a shared thematic and methodological framework is crucial for realizing a global One Health disease surveillance approach [84]. This framework would necessitate the integration of diverse datasets, ranging from genomics to environmental metrics, to transform complex information into actionable insights in a timely manner [84].

Conclusion

The exploration of the fecal virome at the human-animal interface is not yet a proven component of pandemic preparedness—the evidence base remains too nascent, fragmented, and methodologically heterogeneous to support operational implementation. However, we argue that this reflects the field's infancy, not its irrelevance. The convergence of three factors positions fecal virome research as a priority for future pandemic prevention architectures: (1) the documented role of animal reservoirs in past emergences, (2) the recognition that most zoonotic viruses spend part of their ecology in the gastrointestinal tract, and (3) the maturation of metagenomic technologies enabling increasingly comprehensive viral discovery.

The goal is not to replace existing surveillance—clinical sentinel systems, wastewater monitoring, targeted veterinary surveillance—but to add a new layer: **pre-emergence intelligence at the human-animal interface**. By systematically mapping viral diversity where humans and animals intersect most intensely, we can identify viral lineages with zoonotic potential before they cause human disease, prioritize them for experimental risk assessment, and inform targeted interventions. This shifts the paradigm from reactive outbreak response to proactive risk stratification.

Achieving this vision requires the coordinated research agenda we have outlined—longitudinal cohorts, methodological standardization, functional studies, and diagnostic translation—implemented with global equity. The uncharted frontier of the fecal virome will not yield its secrets easily, but the potential reward—a genuinely predictive approach to viral emergence—justifies the investment.

A comprehensive understanding of circulating zoonotic agents, their hosts, vectors, and environmental sources is essential for developing effective integrated One Health monitoring, prevention, and control strategies.

Abbreviations

COVID-19	Coronavirus Disease 2019
CPM	Counts Per Million
CRISPR	Clustered Regularly Interspaced Short Palindromic Repeats
dPCR	Digital PCR
ECoV	Equine Coronavirus
HCV	Hepatitis C Virus
HEV	Hepatitis E Virus
IBD	Inflammatory Bowel Disease
mNGS	Metagenomic next-generation sequencing
PCR	Polymerase Chain Reaction
PEG	Polyethylene Glycol
qPCR	Quantitative PCR
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2
VLP	Virus-Like Particle
WOAH	World Organization for Animal Health

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