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NANOPORE SEQUENCING IN VETERINARY HEALTH SCIENCES: A REVIEW

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ABSTRACT

In veterinary health, the ability to perform field-portable, real-time long-read sequencing, which is unattainable with traditional platforms, makes nanopore sequencing one of the most revolutionary tools. In recent years, the use of nanopore sequencing technology in veterinary medicine has gained significant attention focusing on pathogen detection, antimicrobial resistance (AMR) profiling, livestock genetic identification, microbiome studies, and disease monitoring in wildlife and domesticated animals. Its usefulness in field diagnostics and outbreak response is invaluable in remote resource-limited region because it is portable and can deliver results in a short time. Despite the obstacles of higher error rates and the need for bioinformatics skills, chemistry, base calling algorithms, and workflow of nanopore sequencing are improving its precision and accessibility. In this paper, we review the existing literature on nanopore sequencing technology, its veterinary applications, advantages over other sequencing methods and propose integrated One Health strategies for the future. As presented in the review, technology offers prospective opportunities that could improve animal health, food safety, and monitoring of zoonotic threats in the context of humans, animals, and environment.

Keywords: Nanopore sequencing, Oxford Nanopore Technologies, veterinary genomics, pathogen detection, antimicrobial resistance

INTRODUCTION

Improvement in the scientific methods of DNA sequencing has revolutionized the field of biological science research (Ji et al., 2024). Introduction of long-read systems, particularly single-molecule sequencing systems has changed the long-ruled sequencing methods such as Sanger sequencing or short-read systems (Kumar et al., 2024). Oxford Nanopore Technologies (ONT) has led the development of portable devices that can sequence the DNA or RNA as they pass through the nanopores in a device (Chen et al., 2023). With no prior PCR amplification, these new types of sequencers provide real-time, in-situ data processing and have ultra-long reads (Koivunen, 2019). Nanopore sequencing has gained attention in the field of veterinary genetics due to its in-field pathogen surveillance ability (Santos et al., 2020). Present article aims to review the applications of nanopore sequencing technology in the field of veterinary health science.

Principles and Mechanisms of Nanopore Sequencing

Nanopore technology operates based on DNA or RNA molecules being threaded through nanoscale protein pores deeply set within/surrounded by and part of a membrane (Ying et al., 2022). The signal processing schemes perform more complex interpretations to form base calls from patterns of unique ionic current disruptions generated by each nucleotide entering the pore. With the ONT platform, base calls generated by Guppy and Dorado neural networks can give meaning to sequences captured by the raw signal. With improved base calling models and newer chemistries (R10.4, Q20), the accurate raw readout has been boosted to over 99 percent as reported in (El-Lagta et al., 2024). Specifically, nanopore sequencing reads native molecules: DNA alterations such as methylation are preserved and can be accessed during the

sequencing process (Gouil and Keniry, 2019). With varying degrees of portability from fully portable MinION and Flongle to high-output PromethION, ONTs can also be designed to allow adaptive sampling where sequencing reads are altered dynamically based on the user's specifications for enrichment and rejection in real-time (Pugh, 2023). Thus, the core nanopore mechanism single-molecule, long-read, real-time sequencing is highly beneficial to veterinary medicine.

Applications in Veterinary Health Sciences Pathogen Detection and Surveillance

Within a short period, nanopore sequencing has been applied for the identification and genomics characterization of animal pathogens. For example, during outbreaks of foot-and-mouth disease (FMD), the MinION portable sequencers allowed on-site sequencing and processing of the FMD virus. Brown et al. (2021) demonstrated that any MinION could produce a full-consensus FMDV genome (serotypes A, O, Asia-1) within minutes and cross-referenced via Illumina for instant serotype characterization, yielding 100% overlap for consensus FMDV genome (Brown et al., 2021). Tick-borne zoonoses surveillance experienced the same benefit, such as in a metagenomic nanopore study of Ethiopian livestock ticks where the field isolates of *Rickettsia*, *Francisella*, *Spiroplasma*, *Ehrlichia*, *Borrelia*, a *Babesia* parasite, and even a poxvirus were detected (Chadd et al., 2025). Another advance has been in detection of pathogen in ecological systems Telussa et al. (2025) using MinION for environment poultry waste pathogen surveillance revealed 98% sufficiency in known resistance genes and 96% inferable virulence in comparison to reference assemblies (Telussa et al., 2025). The portability and speed of nanopore sequencing makes it possible to track and perform real-time One Health surveillance from livestock to wildlife for tracking outbreak detection.

Antimicrobial Resistance (AMR) Profiling

Nanopore sequencing technology is capable of rapidly profiling antimicrobial resistance (AMR) genes in samples from animal associations (Slizovskiy, 2024). The long reads are likely to span entire resistance loci, plasmids, facilitating the connection through mobile genetic elements. The MinION was utilized in the Indonesian slaughterhouse study to confirm the identification of 98 % of the known AMR genes, including phenotypic resistance prediction at 91 % concordance to laboratory tests (Telussa et al., 2025). These results outperformed those generated by Illumina MiSeq in alignment with the hybrid-reference-Illumina detected 95% of ARGs. Moreover, with nanopore sequencing, entire bacterial or metagenomic genomes can be uncultured sequenced, enabling the discovery of novel resistance genes and their genomic loci. For instance, real-time nanopore sequencing was used during rapid AMR profiling during bloodstream infections, thus, enabling its use for in-field veterinary diagnosis. In conclusion, these studies prove the importance of veterinary AMR surveillance through the use of nanopore technology, enabling comprehensive resistome analysis at unprecedented speeds and from local (ONT, 2023).

Livestock Genetics and Breeding Programs

In livestock genomics, there has been a groundbreaking development in long reads with nanopore sequencing. The accumulation of ONT data has been used to generate covering animal genomes as well as to identify structural variations of specific traits. One of the most recent projects is the bovine genome project where ONT was used to provide telomere-to-telomere bovine chromosomes assemblies. ONT ultra-long reads were used to achieve very high consensus accuracies of ~Q51 (99.999%) (Li et al., 2023). This accomplishment illustrates the outstanding

capability of nanopore technology to decipher highly complex immune-gene aggregate sequences and the structural variations which are impossible to achieve on short reads. Lowpass nanopore sequencing has been applied to genotype imputation to make genotyping by sequencing more efficient, especially in breeding. Lamb et al. (2023) showed that 0.1x coverage ONT reads, when imputed with densely spaced SNP panels, not only demonstrated high-correlation (>0.91) with genomic estimated breeding values (GEBVs) of the SNP-chip values, but also low-coverage reads which reflected diversity in breeding were shown to have high genomic estimate of breeding value (>0.92) compared with other highly correlated (>0.85) SNP-chip values (Lamb et al., 2023). Research has indicated that a significantly reduced coverage of 0.1x ONT reads, approximately 5 million reads, can yield accuracy in GEBV that is comparable to, or even surpass, that provided by low-density SNP chips. The inherent characteristics of nanopore technology facilitate the generation of reads that overlap multiple genetic markers, thereby contributed to enhanced phasing efficiency and improved imputation capabilities. Consequently, the application of genotype level analysis utilizing nanopore sequencing is deemed feasible in practical agriculture settings. For instance, in a controlled experiment involving the MinION workflow, an average through imputations, achieving a correlation of 0.92 with trait predictions that employed SNP chip-based methodologies. This level of accuracy was attained utilizing portable sequencing systems, demonstrating the practical applicability of this technology in the field of genomics (ONT, 2024).

Microbiome Studies (Nutrition, Immunity, and Disease)

The influence of microbiomes on animal nutrition, immunity, and health permeates even into nanopore sequencing. Its ability to

read long reads facilitated higher resolution taxonomic sequencing of entire 16S rRNA and some other marker genes which enabled the analysis of bacterial strains. With MinION, the pig gut microbiome was sequenced by producing 4.8 million long reads with an average length of 1.7 kb. Using MinION sequencing, 214 bacterial genera were identified as compared to 183 with Illumina data (Tort-Miró et al., 2025). Measures of microbiome diversity were highly correlated at the broader taxonomic levels, yet some discrepancies at the genus level still accounted for remain (Tort-Miró et al., 2025). Both nanopore and Illumina systems captured key community patterns (high numbers of Firmicutes in the microbiota of healthy sows) (Son et al., 2024). The influence of age in companion animals was illustrated in a study where dogs were Illumina/Nanopore sequenced. The study showed that the beneficial gut genera decline with age in dogs, while pathogenic genera increase. Such examples highlight why some draw attention to long read result being comparable to short read results (r0.96 on phylum) while giving full length operon/amplicon data (Tort-Miró et al., 2025). Furthermore, nanopore technology can be used to meta-transcriptomics or direct RNA sequencing of the microbiome, which ONT refers to as providing insights into the “working of microbes”. The real-time and long-read metagenomics enabled by ONT promise to be powerful in examining the microbiome of animals in relation to their diet, infection tendencies, and immune condition.

Wildlife and Conservation Genetics

Conservation genomics nanopore sequencing is another area of focus. The affordability and field-portability of technology make it possible to sequence endangered species without collecting and sending samples. One of the most complete marine mammal genomes, the hourglass dolphin, was sequenced using a single flow cell, and under 10 percent of the cost that is typically used. This was done using a PromethION run on a laptop. The portable MinION enabled the first fully on-site assembly of the red-fronted brown lemur genome in Madagascar. These advances greatly reduce barriers to low-resource settings (ONT, 2024). Adaptive nanopore sequencing also performed well for environmental monitoring, as demonstrated in Aotearoa where New Zealand soil samples were sequenced for distinct, expertly endangered kakapo genotypes, promising non-invasive monitoring of wildlife (Urban et al., 2023). Rapid field-deployable sequence can leverage wildlife forensics for anti-trafficking purposes by identifying species in biological samples quickly (Alketbi, 2024). In total, conservation genomics entering a new stage because of nanopore tools that provide access—even in compliance with the Nagoya Protocol—and facilitate One-Health stewardship by the locals (Urban et al., 2023).

Previous Research in Veterinary Science Applications of Nanopore Sequencing

Table 1 summarizes the key studies that have applied nanopore sequencing in various areas of veterinary science, including pathogen detection, genomic surveillance and antimicrobial resistance profiling.

Table 1 Nanopore Sequencing research conducted in Veterinary Health Sciences (2019–2025)

Country	Study site (setting)	Species Studied	Pathogens studied or microbiome focus	Reference
Belgium	Field & diagnostic cattle (Belgian herds)	Cattle (calves)	<i>Mycoplasma bovis</i> (resp. pathogen), antimicrobial resistance markers (via Nanopore GWAS)	Bokma et al. 2021
USA	Dairy herd / proviral DNA diagnostics	Cattle (dairy herd)	<i>Bovine leukemia virus</i> proviral genome sequencing and transmission analysis	Pavlisca et al. 2021 es New Roman
Spain	Canine gut faeces	Dogs	Microbiome: high-quality metagenome-assembled genomes (MAGs), ARGs, prophages & plasmid linkages	Cuscó et al. 2022
Türkiye	Tick pools from Anatolia	Ticks (field-collected)	Bacterial pathogens in infections; same-day ID via metagenomic Nanopore plus AMR prediction	Ergunay et al. 2023
Mongolia	Grazing ruminant tick surveillance	Ticks	Bacterial (<i>Rickettsia</i> spp., <i>Coxiella burnetii</i> , <i>Borrelia</i> , <i>Anaplasma</i>), viruses (Alongshan, Beiji nairovirus), <i>Theileria</i> , <i>Babesia</i>	Ergunay et al. 2024
UK	Canine clinical cases (urine/skin)	Dogs	Pathogen detection; AMR profiling	Ring et al. 2023~
Poland/Bulgaria	<i>Ixodes ricinus</i> & <i>Dermacentor reticulatus</i> pools	Ticks	<i>Rickettsia</i> spp. (e.g. <i>R. asiatica/raoultii</i>), <i>Neoehrlichia mikurensis</i> , <i>Anaplasma phagocytophilum</i> , <i>Coxiella burnetii</i>	Nelson et al. 2024
South Africa	Tick genomics and endosymbiont assembly	Ticks	Tick genome assembly & <i>Coxiella</i> -like endosymbiont characterization	Meiring et al. 2025
Bhutan	Canine-borne pathogen surveillance (One Health study)	Blood-borne vectors (ticks/ fleas)	<i>Mycoplasma haemocanis</i> , <i>Ehrlichia canis</i> , <i>Anaplasma platys</i> , <i>Bartonella</i> ,	Huggins et al. 2024
Spain	Swine farms – gut microbiome (sows & piglets)		Gut bacterial diversity; potential pathogens (<i>Escherichia</i> / <i>Shigella</i>) vs. beneficial taxa (<i>Lactobacillus</i> etc.)	TortMiró et al. 2025
Japan	Mastitic bovine milk samples		Bovine mastitis pathogens (<i>E. coli</i> , <i>Strep. uberis</i> , <i>K. pneumoniae</i> , <i>S. aureus</i>) identified via 16S rRNA Nanopore in ~6 h	Usui et al. 2023
Saskatchewan feedlot, Canada (chronically ill cattle)	Nasopharyngeal swabs of cattle with unresponsive respiratory disease	None	<i>Moraxella bovoculi</i> , <i>Mannheimia haemolytica</i> , <i>Mycoplasma dispar</i> , <i>Pasteurella multocida</i> (BRD bacteria + ARGs)	Freeman et al. 2022
Jena, Germany (Federal Salmonella reference lab)	Isolated <i>Salmonella enterica</i> from bovine outbreaks	None	Multiple <i>Salmonella enterica</i> serovars + genetic markers for antimicrobial resistance & virulence	Thomas et al. 2023

Country	Study site (setting)	Species Studied	Pathogens studied or microbiome focus	Reference
Nebraska Veterinary Diagnostic Center, USA	Tissue / serum samples from sick pigs	None	Mixed viruses: bovine viral diarrhea virus, bovine herpesvirus1, influenza A virus, Seneca Valley virus, etc.	Neujahr et al. 2024
Le Crotoy wetlands, France (wild poultry surveillance)	Cloacal/tracheal swabs from domestic birds and wild waterfowl	None	Highly pathogenic avian influenza A(H5N1) (whole genome)	Croville et al. 2024
Kandy District, Sri Lanka (dairy farms)	Cow dung/env. samples from dairy production sites	None	<i>Enterobacter cloacae</i> complex (multidrugresistant; blaNDM, blaCTXM, etc.)	Kumari et al. 2025
Wisconsin, USA (dairy cattle surveillance)	Farms affected by the spillover of HPAI from dairy cattle	None	Highly pathogenic avian influenza A H5N1 (metagenomic & targeted)	Caserta et al. 2024
St. Lawrence Estuary, Canada (marine wildlife virology)	Pinniped necropsy samples from seals	None	HPAI H5N1 wholegenome sequences (GridION)	Lair et al. 2024

Comparative Advantages of Nanopore Sequencing

Nanopore techniques, as with other technologies, come with several advantages over the traditional techniques. Unlike short-read platforms, the ONT’s long reads surpass short read platforms by capturing entire repeat sequences, structural variants, and full genes (Kaplun et al., 2023). Consequently, this provides extensive genome assemblies as well as long-read haplotypes phasing. An example of ONT’s advantages includes the construction of gapless assemblies of cattle chromosomes in the part of the immune loci (Li et al., 2023). Devices like MinION and Flongle can be transported to any location with Internet access and can even be used on a smartphone with SmidgION. Unlike laboratory-based sequencers, which take days to provide results, farm and clinic samples can be analyzed on spot. Sequencing results are delivered instantly, enabling immediate

data analysis. Unlike other techniques, this approach does not involve PCR amplification which reduces bias and preserves encoding (marks). Just as examples of base alterations such as 5mC or m6A, can be noted within the sequencing process without any extra steps being taken (Buytaers et al., 2021), this sorts of shifts aid in the discover of solutions to problems in the regulation of genes as well as epigenetics of viruses which could not be resolved using standard methods. The nanopore device processes a wide range of inputs, including tiny amplicons, cfDNA, long genomic DNA, double stranded DNA, and even native RNA, all in a single workflow on one device (Si et al., 2024). To determine the isoforms, all transcriptomes (cDNA or direct RNA) can be sequenced. In RNA-seq, host DNA can be depleted or enriched through adaptive sampling, so modification does not need to be made to the library. ONT offers one-sample and high-throughput devices (Flongle, GridION, PromethION) (Tytgat,

2022). This flexibility benefits either a single field trial or a complete consortial project. The pricing structure is equally advantageous. A MinION flow cell costs roughly 900\$ and provides ~15-30 Gb, more than enough to sequence bacteria or small eukaryotes, and can often be washed and reused. EPI2ME cloud runs base calling and data analysis using AI/ML on custom proprietary pipelines developed by ONT, applying them through an open preference system. GPU-accelerated on-board base calling and machine-learned variant calling processes raw data into biological data for quicker than ever before. Such efficiencies are particularly beneficial for veterinary research. The capability to phase distance SNPs and perform genomic selection more efficiently is possible through drone-enabled nanopore reads. Moreover, the potential to recapture entire viral genome in a single read is equally impressive (Romagnoli et al., 2023). According to ONT resources, the benefits are described as follows: short reads from 50 bp to over 4 Mb, high quality genomes at any read length with the least contig, and resolved genomic regions beyond the reach of short-read amplicons which are free from amplification bias enabling the detection of base modifications (ONT, 2024).

Limitations and Technical Challenges

As promising as nanopore sequencing is, it certainly has its disadvantages. The raw nanopore read accuracy has been blamed for a $Q < Q25$, resulting in 640 bases errors per bacterial gut-strain genome (Bejaoui et al., 2025). Even with improvements in chemistry chemistry and base caller (R10.4.1, SUP models) claiming over 99% single-molecule precision, there are still challenges in single-read precision tasks that arise from higher rates (El-Lagta et al., 2024). This is why many programs add high coverage together with consensus polishing, most often short-read hybrids, to achieve a reference quality sequence. As an example, Bejaoui et al. (2025) have

shown that greater Nanopore error indicated lesser accuracy in nanopore phylogenies than those generated by Illumina during outbreak tracing (Bejaoui et al., 2025). Some errors due to context have been reported, but dual reader pores (R10) rectify these context errors (Tytgat et al., 2020). When combined with flow cells, MinION output of over 10-30 Gb of data per run makes it less efficient for large genomes or high depth metagenome projects. Smaller ONT devices attempt to solve some of these problems but come at greater cost and infrastructural need. Long-read libraries might need other requirements as more-molecular-weight DNA like well-prepped libraries may pose very low-input and heavily degraded samples. Some devices for protein nanopore experiments have a very short shelf life and with use, can wear out after a few dozen hours of runtime. (ONT, 2023). Nanopore data analysis requires a strong bioinformatics background, particularly with base calling, alongside high computational power. The evolution of pipelines is coming too rapidly, which makes it hard to keep up.

CONCLUSION

In a short period of time, these technologies have become an essential asset in veterinary health sciences. This tool enables new pathogen detection and large-scale animal genomics and microbiome characterization, thanks to its long reads, real-time data processing, and field probability. The advantages of nanopore technology are already being harnessed with complete livestock genome assembly and onsite breeding stock genotyping. As with other technologies, nanopore faces limitations relating to error rates and throughput, which are being gradually resolved with enhanced hardware and software. Importantly, nanopore technologies align with the One Health paradigm as they integrate human, animal and environmental genomics. With nanopore tools, *In-Situ* Lab and similar multidisciplinary

projects could empower local professionals to perform genomic surveillance, while providing remote oversight.

CONFLICT OF INTEREST

The authors declared that there is no conflict of interest.

CONTRIBUTION

Conception: RMKS, MH; Design: SA, HA; Supervision: RMKS, MH; Materials: NH, ED; Data Collection and/or Processing: HA; Analysis and/or Interpretation of the Data: RMKS, MH; Literature Review: SA, NH, ED; Writing: RMKS, MH, HA; Critical Review: HA

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SEKVENCIRANJE POMOĆU NANOPORA U VETERINARSKIM ZDRAVSTVENIM NAUKAMA: PREGLED

SAŽETAK

U veterinarskoj medicini, mogućnost izvođenja terenskog, realnog sekvencioniranja dugih nizova u stvarnom vremenu, nije bilo moguće sa tradicionalnim platformama, a što čini nanopore sekvencioniranja jednom od najrevolucionarnijih tehnologija. U posljednjim godinama, upotreba nanopore tehnologije u veterinarskoj medicini privukla je značajnu pažnju, s fokusom na detekciju patogena, profilisanje antimikrobne rezistencije (AMR), genetsku identifikaciju stoke, istraživanja mikrobioma i praćenje bolesti kod divljih i domaćih životinja. Njena korisnost u terenskoj dijagnostici i odgovoru na epidemije je neprocjenjiva u udaljenim i resursima ograničenim područjima, jer je prenosiva i može pružiti rezultate u kratkom vremenu. Uprkos izazovima poput veće stope grešaka i potrebe za bioinformatičkim znanjem, hemija, algoritmi za bazno očitavanje i radni tok nanopore sekvencioniranja stalno se poboljšava, čime se povećava preciznost i dostupnost.

U ovom radu pregledali smo postojeću literaturu o nanopore tehnologiji, njenoj veterinarskoj primjeni, te istakli prednosti u odnosu na druge metode sekvencioniranja i predložimo integrisane strategije u okviru koncepta Jedno zdravlje (One Health) u budućnosti.

Kako je prikazano u radu, tehnologija nudi perspektivne mogućnosti koje mogu unaprijediti zdravlje životinja, sigurnost hrane i praćenje zoonotskih prijetnji u kontekstu ljudi, životinja i okoliša.

Ključne riječi: Nanopore sekvencioniranje, Oxford Nanopore Technologies, veterinarska genomika, detekcija patogena, antimikrobna rezistencija