A Comprehensive Exploration of Microfluidics in Precision Livestock Farming for Disease Detection and Management

Anita Sharma
Agriculture and Forestry University, Nepal
anita.sharma@afu.edu.np

Rajesh Thapa
Department of Veterinary Medicine and Animal Science, Tribhuvan University, Nepal
rajesh.thapa@iaas.tu.edu.np

Abstract

Precision livestock farming utilizes advanced technologies to monitor the health and wellbeing of livestock. Microfluidics, the science of manipulating and controlling fluids in miniaturized devices, shows immense promise for the early detection and management of diseases in livestock. This paper provides a comprehensive review of the current and emerging applications of microfluidics in precision livestock farming, specifically for disease diagnosis and management. An overview of microfluidics fundamentals and key techniques such as lab-on-a-chip, organ/body-on-a-chip, and point-of-care diagnostics pertinent to the livestock industry is provided. Detailed insights into the use of microfluidics for detection of key livestock diseases affecting cattle, poultry, swine and aquaculture are presented. Future perspectives on the integration of microfluidics with other emerging technologies including nanotechnology, synthetic biology and AI/ML for predictive health monitoring and rapid response are discussed. Overall, this paper underlines the significant benefits microfluidic biosensors could provide for early disease diagnosis, continuous health monitoring, and data-driven management decisions in livestock production.

Keywords:

- Microfluidics
- lab-on-a-chip
- organ-on-a-chip
- digital microfluidics
- point-of-care diagnostics
- lateral flow assays

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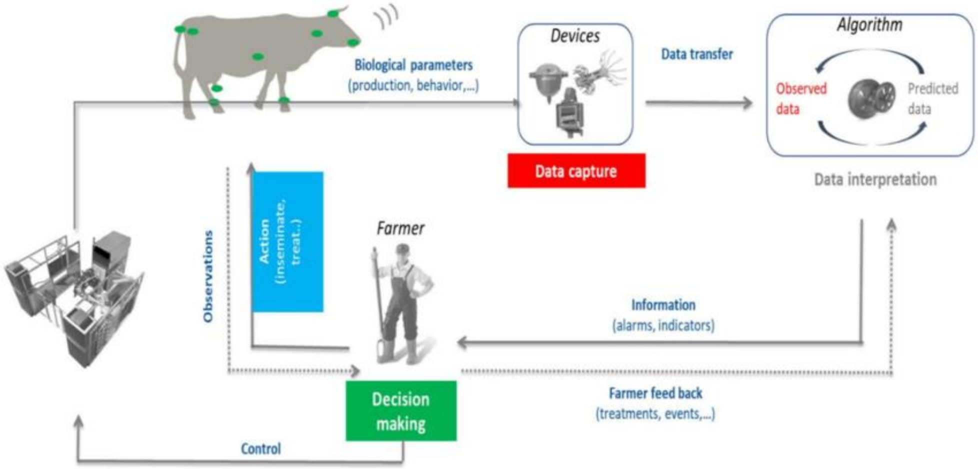
Introduction

The exponential growth of the global population, expected to reach over 9 billion by 2050, has highlighted the critical need for sustainably enhancing food production capacities, especially livestock-derived proteins for human nutrition. Animal agriculture currently provides 18% of global caloric consumption and 33% of protein intake, including meat, milk, eggs and aquatic foods. However, analysts project a 70-100% increase in livestock yields is essential over the next few decades to fulfill escalating nutritional demands driven by rising incomes, urbanization and diet diversifications across developing nations. Animal farming also plays pivotal socioeconomic roles in providing livelihoods and nutritious, affordable food access to over 1 billion smallholder producers worldwide [1]. However, realization of the full potential of livestock sectors in equitably driving food security and rural prosperity is severely stymied by animal disease burdens globally [2].

Figure 1.

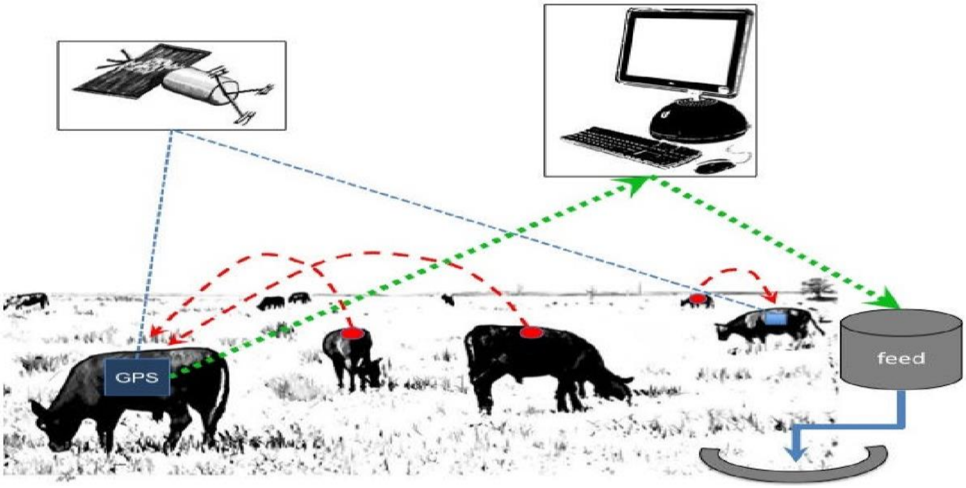


Infectious diseases remain the most impactful challenge undermining the productivity, efficiency and welfare of livestock farming worldwide. Endemic and emerging animal disease epidemics are occurring with increasing frequency, virulence and magnitudes amidst intensifying livestock production systems and globalization trends aiding infectious pathogen dissemination across national borders and trading networks [3]. Over 200 livestock diseases have been identified which can cause devastating clinical symptoms and mortality rates in affected herds [4]. Transboundary animal diseases alone are responsible for 2.5 billion deaths annually across cattle, swine, poultry and ovine herds, causing over $200 billion in economic damages through losses of diseased animals and control expenditures. In addition to direct losses to producers, animal disease outbreaks disrupt regional and global meat/dairy supplies eventually impacting availability and affordability of essential foods [5], [6].

Zoonotic animal pathogens also pose immense threats to human health, as evidenced during recent epidemics of avian influenza, Rift valley fever, Nipah virus and others. Furthermore, foodborne illnesses arising from Salmonella, Campylobacter and other livestock-associated bacteria annually sicken over 600 million consumers worldwide. Rising antimicrobial resistance propagation into human pathogens due to extensive veterinary usage also urgently necessitates optimization of antimicrobial applications in animal farming [7]. This multifaceted burden of animal infectious diseases on livestock economies, rural livelihoods, nutritional security, ecosystem health and public health signifies the need for transformative approaches to safeguard animal health and resilience worldwide [8].

Conventional livestock disease management relies extensively upon veterinary examination of infected animals to diagnose illness based on clinical manifestations, followed by treatment administration or emergency vaccination campaigns. However, many endemic livestock infections persist in herds as inapparent subclinical infections which cannot be visually identified and acted upon. By the time overt disease is detected, pathogens may have already propagated unchecked through entire production facilities and surrounding ecosystem contacts [9]. The lag between infection onset and subsequent diagnosis leads to delayed treatment which enables rapid emergence and mortality during disease outbreaks. Additionally, confirmatory diagnosis is heavily dependent upon extensive laboratory testing which requires time-consuming protocols, expansive infrastructure and technical expertise limiting adoption in rural livestock environments. While molecular and serological diagnostic capabilities have certainly advanced veterinary epidemiology capabilities significantly, dependence upon testing blood, swabs, tissues or carcasses pose practical challenges around invasive sample access and preservation during transport to centralized laboratories [10]. These limitations around subjective or resource-intensive livestock diagnostic approaches constrain effective disease control and obfuscate understanding of infection epidemiology [11].

Figure 2.



Precision livestock farming has recently emerged as a promising paradigm to transform data-driven animal health monitoring by harnessing advanced digital technologies, quantitative analytics and interconnected systems [12]. It focuses on continuous collection of physiological, behavioral or production parameters from individual animals that canmanifest subtle but predictive deviations from normality during early stages of infection or illness onset. Automated health monitoring enables objective identification of at-risk animals for timely intervention even prior to emergence of visually detectable clinical symptoms [13]. As such, precision health technologies deliver practical solutions to overcome subjective limitations of visual veterinary appraisals for infectious disease control. Intelligent analysis of real-time biomarkers also facilitates data-informed decisions on optimal application of vaccines, antimicrobials and biosecurity measures. Precision diagnostics integrated into routine production workflows allows consistent surveillance over the entire animal lifecycle rather than sporadic snapshots. However, realizing the extensive benefits of precision livestock farming requires diagnostic platforms meeting key attributes - rapid analysis, ease-of-use, affordability and suitability for farm working conditions.

Microfluidics offers an extremely promising technological domain which can fulfill diverse precision diagnostic gaps experienced in livestock production settings. The current review provides extensive insights into microfluidic mechanisms, devices and applications which can transform point-of-care animal disease detection capabilities to strengthen livestock health systems worldwide [14].

Fundamentals of Microfluidics

Microfluidic biochips comprise miniaturized assays, sensors and processes integrated to manipulate fluids geometrically constrained within channels measured in tens to hundreds of micrometers. Also referred to as lab-on-a-chip (LOC) or organ/body-on-a-chip, these nimble platforms automate intricate laboratory operations within diminutive credit card-sized cartridges for expedited in vitro testing. Micropumping mechanisms precisely maneuver diminutive liquid volumes through onboard microchannel networks towards segmented reaction chambers, Partitioned sample plugs undergo mixing, separation, dilution, labeling, heating and detection via integrated microvalves, micromixers, microheaters and microarray sensors [15]. Microfluidic biochips can replicate multi-step sample processing and molecular testing workflows including nucleic acid isolation, amplification, sequencing, immunoassays, mass spectrometry and cell culture assays traditionally confined within centralized laboratories. The field of microfluidics harnesses intrinsic properties unique to microscale fluid dynamics for enhancing reagent transport, reaction kinetics and analytical sensitivity [16].

Table 1: Representative microfluidic devices for livestock disease detection

Livestock	**Target Disease**	**Sample Type**	**Microfluidic Technique**
Cattle	Bovine viral diarrhea	Serum	ELISA
Poultry	Avian influenza	Tracheal swab	PCR
Swine	Porcine epidemic diarrhea virus	Feces	Lateral flow assay
Aquaculture	Streptococcus’s	Plasma	Microbead fluorescence immunoassay

Reduced channel dimensions maximize surface area-to-volume ratios for enriched target capture. Diffusional mixing and heat transfer kinetics also heighten considerably within microscopic spaces. Additional microfluidic functionalities like droplets, digital microfluidics (DMF) and hydrodynamic focusing enhance assay efficiency. Million-fold assay partitioning is possible on microfluidic chips using picolitre nanolitre emulsified droplets as individual reaction vessels to facilitate ultrahigh-throughput analysis. DMF electronically choreographs discrete droplets sandwiched between patterned electrodes, enabling precise manipulation absent pumps, channels or valves [17]. Hydrodynamic focusing sheaths sample fluids into narrow core streams for enhanced mixing and separation. Integrated micro-electro-mechanical systems called Micro Total Analysis Systems (µTAS) automate the generation, dispensation, mixing and routing of droplets using embedded sensors and actuators through software codes. Microfluidic large-scale integration (mLSI) can further consolidate thousands of micromechanical and microelectronic components like micropumps, microvalves and multiplexers mimicking very-large-scale electronics integration [18].

Additional salient advantages of miniaturized microfluidic assays over conventional benchtop protocols include significantly condensed reagent consumption and assay turnaround times while upholding test sensitivity and repeatability. Microfluidic devicesmeasure 1–10 cm supporting onboard storage of minute liquid volumes, typically 1–100 µl. Such low sample input requirements mitigate costly reagents and precious biospecimen usage, while co-localization of multiple process modules boosts analytical throughput. Soft lithographic bulk fabrication from inexpensive polymers fosters mass-producible disposable chips mitigating contamination risks. Portability promotes widespread point-of-care usage by untrained users. Microfluidics substances encompass elastomers like polydimethylsiloxane (PDMS) which permit gas permeability and optical transparency well-suited for cell culture and microscopy. Rigid thermoplastics like cyclic olefin copolymer (COC) resist organic solvents during molecular biology protocols with low autofluorescence. Hybrid multilayer lamination adequately combines biocompatible PDMS layers for cellular interfaces with rigid COCs supporting valves and pumps [19].

However specialized instrumentation like precision syringe pumps, voltage controllers and microscopes required for fluid actuation, thermal regulation and assay detection pose obstacles for untrained decentralized testing. Technological refinements must pursue simpler auxiliary-free analyte manipulation techniques and equipment-free detection strategies like colorimetric or smartphone-based analytics. Universal standardized fabrication schemes adopting mainstream continuous-flow manufacturing platforms like injection molding, embossing and 3D printing hold promise for shifting mass production. Economical chip fabrication remains contingent on developing optimally simplified fluidic circuitry minimizing control elements without functionality compromises. Prominent microfluidic geometries include flow-through channels, chambers, mixers, droplets, digital microfluidics (DMF) and hydrodynamic focusing arrangements:

1. Microchannels: Straight rectangular conduits facilitate capillary fluid flows by surface tension. Converging channel segments enable mixing by diffusion. Branched designs allow multiparametric analyses.
2. Microchambers: Microfluidic sample reaction reservoirs with characteristic dimensions between 100 µm to 1 mm. Chamber shape, size and connectedness tailored for different applications like mixing, separation or detection.
3. Micromixers: Integrate complex geometries within confined channels to disturb laminar flows for rapid blending of reagents sheathed side-by-side.
4. Droplet Microfluidics: Generates uniform picolitre to nanolitre emulsion droplets carrying isolated assays dispensed at kilohertz frequencies. Facilitates ultrahigh throughput screening.
5. Digital Microfluidics (DMF): Polarizable liquid droplets are independently manipulated across an array of insulated driving electrodes. Droplet movement electronically choreographed without channels/pumps.
6. Hydrodynamic Focusing: Sheathes sample injected within pressurized centre channel stream by buffer streams on both sides. Diminishes dispersive effects.

Key applications leverage microfluidics for clinical applications like point-of-care diagnostics but utility within veterinary contexts remains relatively underexplored but rapidly emerging. Notable microfluidic techniques applied for livestock disease detection include:

1. Polymerase Chain Reaction (PCR): Exponentially amplifies trace nucleic acids for identifying genomic signatures of pathogens or hosts. Droplet digital PCR partitions samples into thousands of nanoliter droplets for absolute quantification.
2. Lateral Flow Assays (LFAs): Labelled disease-specific antigens/antibodies complexes along striped nitrocellulose membranes for visual qualitative indication. Low-cost application in penside testing.
3. Enzyme-Linked Immunosorbent Assays (ELISA): Immobilized antigen capture antibodies specifically bind target molecules then visualized via enzymatic reactions. Microfluidic integration permits higher throughput and sensitivity.
4. Organs/Body-on-Chips: Microfluidic reconstituted tissue and organ systems emulate physiology for controlled experimental infections to evaluate diagnostics or drug candidates.
5. Lab-on-Chip (LOC): Integrates capabilities like sampling, reagent storage, reaction execution and detection into simplified miniaturized cassettes automated via actuators and software for decentralized usage by untrained farm personnel away from well-equipped wet laboratories.

Thus microfluidic biochips effectively consolidate intricate laboratory sample processing and assay protocols within miniaturized footprint cartridges automatically manipulated by optimized micropumps, microvalves and micromixers integrated via clever configurations [20]. Disposable self-contained operational simplicity allows affordable widespread pen-side adoption even in resource-limited livestock farming contexts by untrained farm workers. Integrating microfluidics with low-cost detection techniques like lateral flow strips or smartphone analytics can further spur decentralized precision animal health monitoring to strengthen real-time data-informed decisions [21].

Applications in Livestock Disease Detection

This section details the current and emerging efforts towards developing microfluidic devices for managing key infectious diseases adversely impacting global livestock industries. Representative examples highlighting essential applications are provided herein.

Cattle Diseases: Bovine respiratory disease (BRD) complexes, mastitis, lameness and Johne’s disease are leading cattle afflictions causing substantial economic damages to dairy/beef sectors. Microfluidics shows immense scope for early screening, prediction and management of these diseases as discussed below:

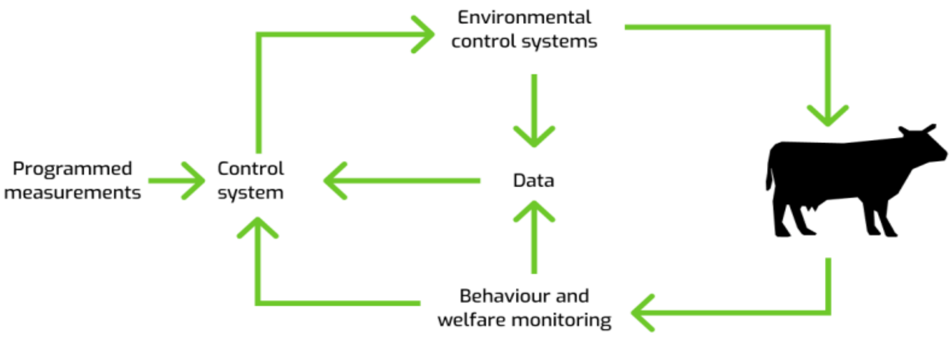
BRD Complexes: Microfluidic ELISA testsdetect pathogen exposure by identifying elevated antibody levels in serum , while PCR chips rapidly identify bacterial/viral nucleic acids in nasal discharge [22]. Integrated LOC devices allow simplified pen-side profiling of multiple infection biomarkers. Additionally, lung/trachea-on-a-chip models facilitate controllable in vitro studies on host-pathogen interactions during BRD pathogenesis investigations [23].

Mastitis: Microfluidic biosensors enable rapid detection of heightened somatic cell counts and pathogen-specific DNA/antigens for predicting/diagnosing mastitis from low-volume milk samples. Disposable microfluidic test strips perform on-site screening within 3 minutes for subclinical mastitis during milking, enabling segregation of infected cows for targeted treatment.

Lameness: Infrared microfluidic sensor strips containing serum can non-invasively detect inflammation biomarkers (e.g. hyaluronic acid) for lameness when adhered onto cattle hooves. Quantifying physiological alterations facilitates early interventions beforephysical manifestations of hoof/leg injuries [24].

Johne's Disease: Microfluidic ELISA and lateral flow strips have been utilized to detect presence of disease-causing Mycobacterium avium subsp. paratuberculosis bacteria in untreated fecal/milk samples within minutes compared to traditional laboratory culture techniques requiring over 6 months. Rapid Johne's diagnosis allows prompt segregation and treatment to limit infection transmission among herds via environmental fecal contamination.

Figure 3.



Poultry Diseases: Poultry farming faces huge losses from viral diseases like avian influenza and Newcastle disease. Additionally, bacterial infections by Salmonella, Campylobacter or Clostridium spp. lead to gastroenteritis transmitted via contaminated poultry products. Microfluidics shows promise for addressing the following poultry disease concerns:

Avian Influenza Virus (AIV): An integrated rotating microfluidic platform performs automatic RNA extraction and multiplex RT-PCR for detection of multiple AIV subtypes H5, H7 and H9 in under 3 hours with high accuracy compared to routine qPCR protocols.

Newcastle Disease Virus: Microfluidic Impedimetric immunosensor chips rapidly quantify antibody levels to identify exposure to Newcastle disease virus from nanoliter serum volumes. This lab-free assay has significant advantages over haemagglutination inhibition tests needing specialized equipment and expertise.

Salmonellosis: Microfluidic electrochemical sensors reliably detect Salmonella Typhimurium contamination in poultry products by targeting phage protein markers derived from specific Salmonella bacteriophages within minutes. Additionally, microfluidic SELEX (systematic evolution of ligands by exponential enrichment) can identify aptamers with high affinity to multiple Salmonella serovars for incorporation into biosensors [25].

Thus microfluidic biochips integrated into poultry supply chain infrastructure can hugely impact infectious disease screening and food safety assurance.

Swine Diseases: Porcine reproductive respiratory syndrome (PRRS), African swine fever (AFS) and foot-and-mouth disease (FMD) are highly contagious and widespread swine afflictions causing serious hardship for pork producers globally. Microfluidics has promising utilities as follows:

PRRS: Microfluidic ELISA chips help quantitate PRRS viral antigen/antibody levels from microliter amounts of plasma, oral fluids or fecal slurry for confirming infection. Nanoparticle-integrated microfluidic devices also detect PRRS viral RNA via fluorescence signaling within an hour to expedite control decisions.

Table 3: Key knowledge gaps inhibiting microfluidics translation

Challenge	**Details**	**Proposed Solutions**
Validation	Limited field testing evidence	Collaborative access to biobanks
Usability	Sample handling complexity	Modular plug-and-play cassettes
Manufacturing	Non-scalable cleanroom fabrication	Mainstream large-scale techniques
Commercialization	User adoption and regulation	Cost-effectiveness demonstrations

ASF: An ultrafast microfluidic mixer has been incorporated into isothermal RT-LAMP assays for detecting African swine fever virus nucleic acid in less than 14 minutes. Result automation using mobile app platforms avoids transmission risks. LDQuartz ® microfluidic assay cartridges utilize silicone photonic sensor arrays to deliver rapid, reliable and portable ASF diagnosis at pen-side.

FMD: Microsphere-based microfluidic devices integrate valveless microfluidics and nanoscale pores tailored for size-selective capture/release of different FMD viral serotypes suspended in samples. This facilitates rapid, efficient concentration and isolation of intact viruses from clinical samples ahead of molecular characterization.

Aquaculture Diseases: Aquaculture provides over half the fish consumed globally but is plagued by heavy losses from water-borne pathogens. Fish biopsies are stressful and difficult to perform routinely. Microfluidics allow continual physiological monitoring using minute samples as follows:

IHNV: Rainbow trout organotypic liver spheroids cultured in specialized microfluidic biochips showed cytopathic effects and death specifically in response to Infectious hematopoietic necrosis virus (IHNV) which infects various farmed/wild salmonids via contaminated water. This liver-on-a-chip mimics pathogenesis for targeted drug testing.

Streptococcosis: Droplet microfluidic ELISA assay integrated graphene oxide nanosheets as electrochemical probes to detect Streptococcus agalactiae antigen in tilapia tissue samples with high sensitivity, allowing species-specific diagnosis. Low sample consumption suits limited volumes available from small fish. Thus, microfluidic organoids or water quality monitors can enable continuous surveillance against frequently emerging aquaculture pathogens [26].

Future Prospects and Conclusions

Microfluidic biosensors demonstrate immense promise for advancing precision livestock farming via rapid, reliable on-site disease diagnosis and real-time health monitoring. However, there are still several challenges that need to be addressed before widespread translation and commercialization of microfluidic devices can be achieved in animal agriculture.

One key limitation is that most microfluidic devices reported in literature have been designed, developed and validated under controlled laboratory conditions using spiked samples. Comprehensive validation using field samples from naturally infected livestock is essential to evaluate sensitivity and specificity under real-world messy environments prior to commercialization. Accessing well-characterized biobanked samples through collaborative efforts will be invaluable to facilitate more rigorous evaluation. Extensive livestock trials are also vital to assess technology robustness under working farm conditions as well as validate health/productivity benefits quantitatively to build end-user confidence [27].

Additionally, further innovation is needed to integrate sample collection, preparation and upstream processing functionalities into microfluidic devices for more seamless point-of-care usage. This will also minimize risks of sample deterioration during handling/transport and procedural errors. Universal pre-analytical sample preparation protocols compatible with diverse detection modalities could allow flexible customization of testing panels for region-specific livestock disease priorities. Lyophilized/stabilized reagents integrated onboard can address reagent instability arising from variable temperatures/humidity on farms. Modular microfluidic cassettes with plug-n-play sensors may also provide flexibility for farmers themselves to measure multiple analytes using affordable standardized instruments [28].

Most reported microfluidic prototypes are currently fabricated using specialized cleanroom microfabrication techniques which can be expensive and non-scalable. Transition towards inexpensive mass manufacturing platforms like injection molding or 3D printing is essential for economical, accessible livestock diagnostics. Quality assurance frameworks also need development to ensure fabrication uniformity across production batches. Additionally, seamless integration with low-cost detection modes like colorimetric assays or camera phones can promote uptake by minimizing reliance upon expensive analyzers. Expanding capabilities via multiplexing or instrument connectivity for data logging/transmission will augment functionalities.

Merging microfluidics with nanotechnologies, synthetic biology and AI/ML also offers avenues to incorporate enhanced sensing capabilities or expand diagnostic panels to rationally guide targeted treatment and control interventions. Implantable livestock biosensors can potentially enable continuous, non-invasive prognosis of infection onset even prior to appearance of microbial or inflammatory markers. Highly distributed on-animal wearables outfitted with miniaturized sensors can provide minute-by-minute flock health indicators to identify environmental risk factors or early behavioral indicators predictive of impending outbreaks. Progressive adoption of microfluidics can massively benefit livestock production via minimizing product losses and promoting animal health/welfare amid rising demands.

This paper provided a detailed yet concise snapshot of the manifold microfluidics applications pertinent in precision livestock farming [29]. The highlighted case studies effectively demonstrated microfluidics' versatility in developing rapid pen-side assays against high-priority livestock pathogens and health biomarkers. Integrated microfluidic tools for predictive smart farming can significantly bolster livestock disease resilience worldwide and support expanded food production [30]. Realization of low-cost user-friendly lab-free diagnostics can be transformative for infectious disease control even in resource-limited livestock farming contexts. However realizing this sustainable impact requires extensive evaluation under field settings and strengthening connections between technology developers and end-users in the animal agriculture sector [31].

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