



Tiny Regulators, Big Possibilities: How MicroRNAs Could Shape Livestock Health and One Health

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Abstract

MicroRNAs (miRNAs) are small non-coding RNA molecules that regulate gene expression by influencing messenger RNA stability and protein production. In livestock, they have been associated with growth, fat deposition, milk production, reproduction, immune responses and disease resistance. Their presence in biological samples such as milk and blood has generated interest in their potential use as non-invasive biomarkers of animal health and physiological status. In dairy cattle, researchers are investigating milk microRNA profiles for their ability to identify subclinical mastitis, a condition that can reduce productivity without obvious clinical signs. Analytical approaches, including reverse transcription quantitative polymerase chain reaction (RT-qPCR), small-RNA sequencing and digital PCR, enable the detection and measurement of these molecules. However, variations related to breed, age, lactation stage, disease status, sample composition and laboratory methods present important challenges. Independent validation, standardized measurement protocols and affordable testing systems are necessary before microRNA-based diagnostics can be adopted routinely. Integrating microRNA profiles with conventional veterinary examinations, genomic information and production records could eventually strengthen livestock health monitoring and support the broader One Health approach.

Keywords- MicroRNAs; Gene regulation; Livestock health; Dairy cattle; Subclinical mastitis; Molecular biomarkers; Bioinformatics; Milk diagnostics; Animal welfare; One Health



It is early morning on a dairy farm. A cow walks into the milking parlour. Her udder looks normal, her milk looks normal, and nothing suggests a problem.

Yet inside her mammary gland, inflammation may already be under way. This is subclinical mastitis: inflammation with no visible change in the udder or the milk, which still quietly lowers milk yield, affects welfare and costs the farmer money. Veterinarians have established ways to look for it, including clinical examination, somatic cell counts, the California Mastitis Test and microbiological culture.

But what if a drop of milk could say more, and sooner? What if a blood sample could reveal stress or metabolic strain in an animal before production begins to fall? These questions have drawn scientists to some of the smallest regulators in biology: microRNAs.

Meet the molecular editors

For a long time, genetics was taught as a simple chain: genes carry instructions, and cells use them to make proteins. We now know that cells must also control *which* instructions are used, *when*, and *how strongly*.

MicroRNAs (miRNAs) are part of that control system [1]. They are only about 20 to 24 nucleotides long and make no proteins themselves. Instead, they bind to messenger RNA (mRNA), the molecule that carries a gene's instructions to the protein-making machinery, and either reduce how much protein is made or help break the message down.

Think of genes as instruction manuals and microRNAs as the editors who decide which chapters get read, which get skipped and which get read twice. One microRNA can influence many genes, and several microRNAs can act on the same gene, so together they form networks rather than simple on-off switches. They have been implicated in growth, metabolism, immune responses and tissue development, and have now been identified in cattle, buffaloes, sheep, goats and pigs.

How a microRNA is made, and how it works

The journey from DNA to a working editor has five steps [1]:

1. **Transcription.** RNA polymerase II copies a microRNA gene into a long primary transcript, the *pri-miRNA*, which folds into one or more hairpins.
2. **Nuclear processing.** The Drosha–DGCR8 complex (the "Microprocessor") trims each hairpin into a *pre-miRNA* of roughly 70 nucleotides.
3. **Export.** Exportin-5 carries the pre-miRNA into the cytoplasm.
4. **Dicing.** The enzyme Dicer cuts off the loop, leaving a short double-stranded RNA about 22 nucleotides long.
5. **Loading.** One strand, the *guide*, is loaded onto an Argonaute protein inside the RNA-induced silencing complex (RISC). The other strand is usually discarded.

The guide finds its targets through its seed region, nucleotides 2 to 8 counted from its 5' end. The seed pairs with complementary sites, usually in the 3' untranslated region (3' UTR) of a target mRNA. In animals the match is only partial, which is why one microRNA can regulate hundreds of different mRNAs. Once bound, RISC represses translation and recruits enzymes that shorten the mRNA's poly(A) tail, leading to its degradation [1].

This regulatory role matters in livestock, where milk yield, growth, meat quality, reproduction and disease resistance all emerge from interactions among genes, physiology and environment. MicroRNAs are catalogued in miRBase under species prefixes such as *bta-* for cattle (*Bos taurus*), *oar-* for sheep, *chi-* for goats and *ssc-* for

pigs, so a name like bta-miR-148a tells you both the molecule and the species.

From milk production to disease resistance: four areas of research

1. Growth, fat deposition and production traits

MicroRNAs have been associated with fat cell development, lipid metabolism and the differentiation of muscle and other tissues. In sheep, miR-148a has been studied for its role in the multiplication and maturation of fat cells. In cattle, miR-149-5p has been examined in bovine fat cell development. Such findings help explain why animals differ in body composition and production traits, and one day integrating microRNA data with genomic information and conventional breeding records could add insight into economically important traits.

A caution belongs here. Finding that a microRNA is *associated* with a trait does not prove that it *causes* the difference. Functional studies and validation in larger animal populations remain essential.

2. Milk production and mammary gland health

The mammary gland changes dramatically during pregnancy, lactation and the dry period. MicroRNAs have been investigated for their part in mammary cell development, lipid synthesis and the regulation of genes linked to milk production.

Milk is especially interesting because it carries not only nutrients but also microRNAs, some of them packaged in extracellular vesicles, tiny membrane-bound parcels that cells use to communicate [2]. Researchers are studying how this cargo varies with physiological state and udder health, which raises a veterinary question: could changes in milk microRNAs eventually help identify changes in an animal's health?

3. Could microRNAs help detect mastitis earlier?

Mastitis, inflammation of the

mammary gland, reduces milk production, affects welfare and creates economic losses. It is hardest to catch in its subclinical form, which is exactly where an extra signal would help.

A 2020 study compared milk from cows with different mastitis statuses and found differences in the levels of several candidate microRNAs. The researchers also explored whether these could be used alongside existing indicators to classify milk samples [3]. Broader reviews are now mapping how non-coding RNAs behave in bovine mastitis [4].

The appeal is practical: milk can be collected repeatedly and without invasive procedures. But candidate biomarkers must prove themselves across breeds, farms, pathogens and management systems before anyone can rely on them. For now, microRNA testing is a promising research direction, not a replacement for established diagnostics.

4. Stress, metabolism and animal welfare

Researchers are also exploring whether circulating microRNAs can reflect physiological stress, metabolic change and other aspects of animal health [5]. Such indicators could eventually supplement behavioural observations and established clinical assessments, giving a more objective picture of how animals are coping with their environment.

Under the hood: measuring microRNAs in milk

Turning "the milk carries a signal" into a usable test is a measurement problem as much as a biological one.

Milk is a mixture of fat globules, skim milk and somatic cells. MicroRNAs in the cell-free skim fraction are unusually stable, because they travel inside extracellular vesicles or bound to Argonaute proteins, which protects them from the RNases that would quickly destroy naked RNA [2]. That stability is a large part of why they attract interest as biomarkers.

Three methods dominate the work:

Method	What it does	Gain
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		limitation
RT-qPCR (often with stem-loop primers)	Measures chosen microRNAs with high sensitivity	Targeted only; needs careful normalisation
Small-RNA sequencing	Discovers and profiles many microRNAs at once	Costly; results depend on library preparation and analysis pipeline
Digital PCR	Counts target molecules without a standard curve	Lower throughput; targeted only

Two issues deserve special attention.

Normalisation. There is no universal "housekeeping" microRNA. Researchers typically add a synthetic spike-in (such as *C. elegans* cel-miR-39) to track extraction efficiency, and use stable endogenous reference RNAs, then report relative expression, for example as fold change from cycle-threshold (Ct) values.

Cell composition. In mastitis, the somatic cell count rises sharply as immune cells flood the milk. A change in a microRNA's level may therefore reflect a different mix of cells in the sample rather than a regulatory change inside the udder's own cells. That does not make the signal useless, but it shapes how it should be interpreted.

A biomarker is judged on its sensitivity, its specificity and its area under the ROC curve (AUC), and these figures count only when they hold up in independent herds that were not used to discover the marker.

What does this have to do with One Health?

One Health recognises that the health of people, animals and the environment is interconnected, and putting it into practice needs veterinarians, doctors, environmental scientists, farmers and public health authorities working together. At first glance,

microRNA research looks like pure molecular genetics. Its relevance becomes clearer when we look at the wider livestock system.

- **Sustainable food production.** Healthier animals support more sustainable production, and a better grasp of animal physiology and disease can inform better monitoring and management.
- **Surveillance.** If reliable molecular indicators of particular diseases are validated, they could complement conventional animal-health surveillance, although the usefulness of any marker depends on the disease and the quality of its validation.
- **Welfare.** Molecular indicators of stress or metabolic strain could supplement behavioural and clinical assessment.
- **Collaboration.** Turning a laboratory finding into a farm tool brings together genomics, diagnostics, epidemiology, animal breeding and data science.

An important distinction must be kept: microRNA research does not directly prevent zoonotic infections or guarantee safer food. Its contribution to One Health is indirect, through a deeper understanding of animal biology and, if validated applications emerge, better decisions about animal health and production.

From laboratory discoveries to the dairy farm

The path from a promising molecule to a farm tool is rarely straightforward. MicroRNA levels can shift with age, breed, stage of lactation, nutrition, environment, disease and even how a sample is handled, which makes universal reference ranges hard to set. Different laboratory platforms and analysis pipelines can also give results that are difficult to compare.

Before a microRNA test can help a veterinarian, it must be accurate, reproducible,



affordable and fast enough for its purpose, and it must hold up in independent validation, because a marker that shines in a controlled study may stumble across diverse farming systems. Cost matters too: a technically accurate test has little practical value if it needs specialised equipment or returns results too slowly.

For India, where dairy farming spans many breeds, production systems and farm sizes, locally relevant validation will be especially important. The goal is not to discover ever more microRNAs. It is to find the few that solve real problems for veterinarians, farmers and the livestock sector.

What lies ahead

The most likely future is not one magic marker but a combination of clues. Researchers can integrate microRNA profiles with genomic data, gene-expression studies, clinical findings, milk-quality measurements and production records. A research programme might test whether milk microRNAs, somatic cell counts and clinical observations together identify subclinical mastitis better than any one alone. Another might ask whether microRNA profiles consistently track heat stress or metabolic disturbance in dairy animals.

Multi-marker panels, combined with statistical classification models such as logistic regression or random forests and validated by cross-validation and independent herds, are more likely to succeed than any single microRNA.

These remain research possibilities, not routine applications. Their worth will depend on rigorous experiments, independent validation and evidence that they improve decisions and outcomes. It also depends on making sure new technologies complement practical veterinary services rather than widening the gap between well-resourced laboratories and smallholder farmers.

The bigger picture: small molecules, shared responsibilities

MicroRNAs remind us that

biological regulation happens at every scale, from ecosystems and herds down to a single cell. They offer a new way to investigate the mechanisms behind livestock growth, production, immunity and disease, and their presence in easily collected samples such as milk and blood has stirred interest in non-invasive ways of monitoring animal health.

Yet scientific progress is measured not only by discoveries in the laboratory but by their usefulness in the field. For veterinarians and veterinary public health professionals, the challenge is to connect molecular findings with sound clinical practice, effective surveillance and evidence-based decisions.

The cow in the parlour still looks perfectly fine. If research delivers reliable and affordable tests, she may one day be understood a little sooner, and these tiny regulators could become one more tool for healthier animals, more sustainable livestock production and the connected goals of One Health. The promise lies not in replacing established veterinary knowledge, but in adding another layer of understanding to it.

Further reading

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