

The Emerging Potential of MicroRNAs as Minimally Invasive Biomarkers in Veterinary Medicine

MicroRNAs (miRNAs) have emerged as one of the most promising classes of minimally invasive biomarkers in animal health. Over the past two decades, research has shown that these small regulatory RNAs are not only remarkably stable in blood and other biofluids, but also closely linked to disease processes. Their potential as biomarkers therefore lies not only in their accessibility, but in their fundamental role in regulating the molecular mechanisms underlying disease.

Traditional biomarkers often reflect downstream consequences of disease, such as protein leakage, tissue damage, or metabolic disturbance. By contrast, miRNAs participate directly in the regulatory networks that drive disease processes. As such, changes in their expression can precede clinical signs, offering the possibility of earlier disease detection. Moreover, because many miRNAs are tissue-specific and exhibit distinct expression patterns in different diseases, they can help pinpoint both the organ affected and the underlying pathological process.

MicroRNAs (miRNAs) are small (typically 20–24 nucleotide), non-coding RNA molecules, that play a critical role in post-transcription regulation of gene expression.¹ MiRNAs were first discovered in 1993, when Victor Ambros and colleagues identified lin-4 in *Caenorhabditis elegans* as encoding a small RNA capable of regulating developmental timing in the nematode, *Caenorhabditis elegans* by base pairing to the 3' untranslated region of lin-14 mRNA.² Initially regarded as a nematode specific oddity, their broader significance became clear with the subsequent discovery of microRNA let-7 by Gary Ruvkun and colleagues, followed by the discovery that this miRNA was conserved across diverse animal species, including mammals.³ This finding established miRNAs as a fundamental, evolutionarily conserved component of gene regulation.

As sequencing technologies advanced through the early 2000s, miRNAs were increasingly recognised as abundant, stable and deeply integrated into the fine-tuning of several biological processes including development, immunity, metabolism and disease pathogenesis. A pivotal turning point came in 2008, when Mitchell and colleagues demonstrated that circulating miRNAs are present and stable in human serum and plasma, despite exposure to conditions that would normally degrade RNA.⁴ This discovery established their potential as non-invasive biomarkers of disease and accelerated interest across biomedical fields.

In human medicine, the feasibility and potential value of miRNA based diagnostics have been demonstrated across multiple disease areas. Circulating miRNA signatures are being actively explored for the early detection of cancer, with studies reporting promising diagnostic performance across case-control and validation cohorts.^{5,7}

In cardiovascular disease, certain miRNAs have been shown to rise rapidly in plasma following myocardial injury and have been proposed as sensitive early markers of acute myocardial infarction. Several studies have reported that cardiac-associated miRNAs can be detected within hours

of symptom onset and may provide diagnostic information comparable with, or in some cases preceding, conventional cardiac biomarkers such as cardiac troponins.^{6–8}

Distinct miRNA profiles have also been associated with autoimmune conditions and chronic inflammatory diseases, such as rheumatoid arthritis, systemic lupus erythematosus and inflammatory bowel disease, as well as exposure to environmental toxins such as tobacco smoke and air pollution. Collectively, these findings illustrate the diagnostic versatility of miRNAs and provide a strong translational foundation for veterinary applications.

Veterinary research in this area is expanding rapidly and already demonstrates clear potential across species. In canine myxomatous mitral valve disease (MMVD), stage-dependent changes in circulating miRNAs have been documented across cross pre-clinical disease and congestive heart failure with several miRNAs showing potential diagnostic and prognostic value.^{10–12} Distinct miRNA signatures have also been described in other companion animal conditions, including dilated cardiomyopathy, lymphoma, osteosarcoma and mammary carcinoma, with studies reporting disease-associated expression patterns and potential diagnostic or prognostic applications.^{13–16} Beyond companion animal medicine, early studies in horses have identified circulating and synovial microRNA profiles associated with osteoarthritis and laminitis,^{17–19} while work in livestock has explored miRNA profiles and their potential as biomarkers of infectious disease, mastitis and mycotoxin exposure.^{20–22}

Collectively, these findings indicate that miRNA-based biomarkers are biologically plausible and analytically feasible across multiple veterinary species. Until recently, however, veterinary studies have focused predominantly on miRNA discovery and characterisation, identifying disease-associated changes in the expression of individual miRNAs or miRNA profiles. Data have largely been generated from single centre studies from small cohorts and focused on specific breeds. Furthermore, challenges such as the need for standardised sample handling and analytical workflows, adequately powered and well phenotyped validation cohorts and demonstration of clear clinical utility alongside existing diagnostics have, to date, limited scope for these findings to be translated into clinical use.

Furthermore, the inherent complexity of miRNA biology adds an additional layer of difficulty to the development of clinically useful diagnostic tests. Individual miRNAs can regulate multiple genes within interconnected regulatory networks, while many are involved in pathways shared across inflammatory, metabolic and neoplastic diseases. Circulating miRNAs may also originate from multiple tissues and occur in different molecular forms and carriers, complicating interpretation of their biological source.^{23–24} Together with inter-individual variation and the limited disease specificity of many individual miRNAs, these factors suggest that diagnostic information may be more reliably captured by multi-miRNA signatures or 'fingerprints,' than by individual miRNAs.²³

The field is now moving into a new phase, in which the use of miRNA signatures as biomarkers of disease is becoming a potentially clinically feasible prospect. The integration

of machine learning and advanced predictive modelling represents an important development in addressing the complexity of miRNA biology. Recent veterinary studies have begun to apply these approaches to a range of companion and production animal diseases. In a multicentre, prospective study of 97 dogs, Palarea-Albaladejo et al. used a 15-miRNA serum panel combined with penalised logistic regression to discriminate dogs with MMVD from healthy controls (AUC 0.93; sensitivity 0.85; specificity 0.82).²⁴ Preliminary data also indicate that miRNA profiles can distinguish cats affected by hypertrophic cardiomyopathy (HCM) from healthy controls (sensitivity 0.89; specificity 0.77; AUC 0.88).²⁵ Further preliminary data indicate that miRNA profiles can distinguish dogs with dilated cardiomyopathy (DCM; n=34) from healthy controls (n=42) (AUC 0.96; sensitivity 0.94; specificity 0.86), and early-stage (stage B) cardiac disease, comprising DCM and MMVD, from healthy controls (AUC 0.98; sensitivity 0.97; specificity 0.79).²⁶ Notably, the panel also differentiated DCM from MMVD despite shared pathological pathways between the two diseases. This suggests that multi-miRNA signatures may capture disease-specific biological differences and could potentially provide information beyond disease detection alone.

Beyond cardiac disease, miRNA expression has also been investigated as a diagnostic biomarker for canine urothelial (transitional cell) carcinoma. When combined with detection of the BRAF mutation, miRNA profiling improved diagnostic performance, achieving an AUC of 0.88, with 0.88 sensitivity and 0.78 specificity.²⁷ Looking ahead, advances in multi-omics integration are likely to broaden the application of miRNA-based diagnostics in veterinary medicine. Combining miRNA profiles with genomic, proteomic and metabolomic data may provide a more comprehensive understanding of disease processes and improve the discriminatory power of biomarker models. These developments may ultimately support precision medicine approaches in veterinary practice, with applications in areas across a variety of diseases, species and contexts.

Much of the emerging veterinary evidence has been generated through work by Mi:RNA, a spin-out from Scotland's Rural College (SRUC), alongside collaborations with academic and clinical groups. Further preliminary studies have explored the potential application of miRNA profiling to canine heartworm disease,²⁸ while ongoing work is investigating the application of miRNA profiling to companion animal chronic kidney disease, anxiety, osteoarthritis and haemangiosarcoma in companion animals.

This work also extends to production animal health, where earlier detection may have implications for animal welfare, productivity and disease control. In a preliminary study of Johne's disease, a chronic and economically significant enteritis of cattle caused by *Mycobacterium avium* subspecies *paratuberculosis* (MAP), Capewell et al. measured 24 circulating miRNAs in serum from MAP positive (n=66) and MAP negative (n=65) cattle and applied machine learning to identify an optimal diagnostic classifier.²⁹ These findings provide an early indication that miRNA profiles may have value in diseases where existing diagnostic approaches are constrained by limited sensitivity, while also demonstrating the applicability of the approach beyond companion animal medicine.

Much of this progress has been made possible by advances in the laboratory methods needed to make miRNA testing reliable and reproducible. Mi:RNA's development and evaluation of workflows for miRNA extraction and detection across multiple sample types, including blood, milk and urine, has provided a foundation for applying these approaches across different species and clinical settings. This cross-matrix

flexibility is particularly important in veterinary practice, where the choice of sample is often determined by species, context and practicality rather than an ideal analytical preference.

Establishing this level of assay robustness and reproducibility has been a critical prerequisite for translating the discovery-phase findings described above into commercially viable diagnostics. It also provides a framework that other groups in the field are likely to adopt as the sector continues to mature. Furthermore, by combining standardised sample-handling and extraction protocols with a proprietary machine learning interpretation model, this work also demonstrates progression beyond single-analyte detection towards a scalable diagnostic platform capable of supporting multiplexed testing.

The recent development of robust platforms capable of measuring multiple miRNAs, alongside machine learning and advanced predictive modelling, has brought their use as disease biomarkers closer to clinical application. The ability to translate this multiplexed approach into rapid, near-patient testing using minimally invasive samples would represent an important milestone in the development of miRNA-based diagnostics, marking a shift from biomarker discovery towards real-world clinical application. These approaches could ultimately allow testing to move beyond centralised laboratories and into veterinary practice, contributing to more accessible, earlier disease detection and monitoring and, ultimately, supporting more informed clinical decision-making across veterinary medicine.

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Dr. Vicky Strong

Dr. Vicky Strong MRCVS is a Clinical Associate Professor at the University of Nottingham School of Veterinary Medicine and Science. She holds a BSc (Hons) in Veterinary Pathology and a Doctorate in Veterinary Medicine (DVetMed). Dr Strong is co-founder of Synapse Animal Health Consultancy, which brings together veterinary and scientific expertise, strategic communication and education, to support organisations working across animal health. Dr Strong works with MIRA as a Consultant in Clinical Education and Engagement in the Business Development team.