Author/s:
Tran, H;McConville, M;Loukopoulos, P

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Metabolomics in the study of spontaneous animal diseases

Helena Tran, Malcolm McConville, Panayiotis Loukopoulos[1]

Abstract. Using analytical chemistry techniques such as nuclear magnetic resonance (NMR) spectroscopy and liquid or gas chromatography–mass spectrometry (LC/GC-MS), metabolomics allows detection of most endogenous and exogenous metabolites in a biological sample. Metabolomics has a wide range of applications, and has been employed in nutrition science, toxicology, environmental studies, and systems biology. Metabolomics is particularly useful in biomedical science, and has been used for diagnostic laboratory testing, identifying targets for drug development, and monitoring drug metabolism, mode of action, and toxicity. Despite its immense potential, metabolomics remains underutilized in the study of spontaneous animal diseases. Our aim was to comprehensively review the existing literature on the use of metabolomics in spontaneous veterinary diseases. Three databases were used to find journal articles that applied metabolomics in veterinary medicine. A screening process was then conducted to eliminate references that did not meet the eligibility criteria. Only primary research studies investigating spontaneous animal disease were included; 38 studies met the inclusion criteria. The main techniques used were NMR and MS. All studies detected metabolite alterations in diseased animals compared with non-diseased animals. Metabolomics was mainly used to study diseases of the digestive, reproductive, and musculoskeletal systems. Inflammatory conditions made up the largest proportion of studies when articles were categorized by disease process. Following a comprehensive analysis of the literature on metabolomics in spontaneous veterinary diseases, we concluded that metabolomics, although in its early stages in veterinary research, is a promising tool regarding diagnosis, biomarker discovery, and in uncovering new insights into disease pathophysiology.

Key words: metabolomics; omics; oncology; One Health; review; veterinary.

Introduction

Metabolomics definition and broad applications

Metabolomics is an emerging "-omics" field aimed toward the comprehensive detection and quantification of metabolites and small molecules in a biological specimen.[18,70] Combining advanced analytical techniques and chemometrics,[49] metabolomics enables researchers to identify a large proportion of metabolites (the metabolome) present in a sample, including amino acids, sugars, ketones, nucleotides, fatty acids, organic acids, microbial metabolites, and exogenous small molecules (including drugs, food additives, and pesticides).[12,31] By analyzing these products of cellular metabolism, metabolomics reveals valuable information about an organism's metabolic or physiologic state at the time of sampling.[11,36]

Metabolomics complements other omics technologies including genomics, transcriptomics, and proteomics, and there are increasing efforts to integrate these different data sets.[77,86] With a tremendously wide range of applications,[42] metabolomics has been previously utilized in environmental analysis,[40] toxicology,[59] nutrition science,[3,75] and systems biology.[74] In food science, metabolomics has been used in conjunction with traditional nutrition assessment methods to identify biomarkers that represent diet-related disease risks.[5] Agricultural and plant science industries have used metabolomics technologies to improve commercially significant traits and increase yield.[26,38]

In the biomedical sphere, metabolomics is being used to identify new disease biomarkers as well as provide novel insights into disease pathogenesis. The identification of endogenous and exogenous metabolites facilitates a better understanding of the complex changes that occur in metabolic and biochemical pathways.[47] Detecting complex changes in metabolite levels can not only aid disease diagnosis,[21] but can also monitor cellular responses to nutrition,[87] drugs,[32] toxins,[35] and environmental factors.[37] Given that

Melbourne Veterinary School, Faculty of Veterinary and Agricultural Sciences (Tran, Loukopoulos), and Bio21 Institute, Metabolomics Australia (McConville), University of Melbourne, Melbourne, Victoria, Australia.

[1]Corresponding author: Panayiotis Loukopoulos, Melbourne Veterinary School, Faculty of Veterinary and Agricultural Sciences, University of Melbourne, 250 Princes Highway, Melbourne Veterinary School, Vet Path, Werribee, Victoria 3030, Australia. panos.loukopoulos@unimelb.edu.au

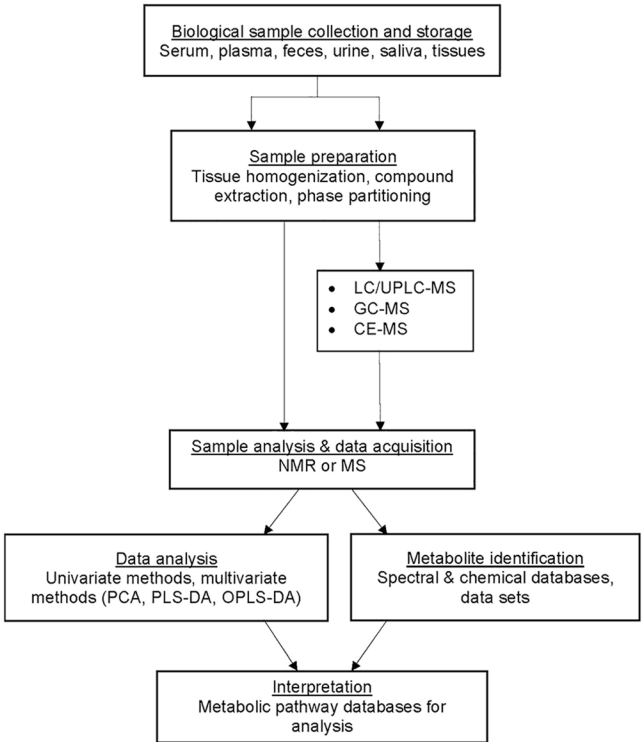

Figure 1. Basic steps of metabolomics studies (based on previous studies,[2,61,69] modified).

altered metabolism is a key feature of cancer, metabolomics is playing an increasingly important role in cancer biology research, with uses ranging from detecting key regulatory molecules involved in carcinogenesis to identifying specific biomarkers for diagnosis.[29,72] In the pharmaceutical industry, metabolomics can identify targets for drug development,[13] assist in mode-of-action studies, and monitor drug metabolism and toxicity.[66]

Analytical techniques

Metabolomic analyses commonly use one or more analytical techniques to facilitate identification and quantification of as many metabolites as possible in a biological sample (Fig. 1).[2,61,69,82] Nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS) are, to date, the most common techniques employed in metabolomic studies.[47,58] One-dimensional NMR spectroscopy is commonly used to detect 10s–100s of metabolites in biological extracts. It is non-destructive (allowing reuse of the sample for other analyses) and can be used to quantify metabolites with high accuracy and reproducibility. Two-dimensional NMR techniques can be used to confirm or elucidate the structures of previously unidentified metabolites, as well as measure the incorporation of stable isotopes in labeling experiments.[43] MS techniques involve the ionization of derivatized or underivatized samples, and detection of corresponding charged ions (as mass-to-charge ratios).[6] MS techniques are generally more sensitive than NMR techniques, and allow greater coverage of metabolites (100s to ~1,000s) in biological extracts.

MS is often coupled with chromatographic separation techniques such as gas or liquid chromatography (GC, LC) or capillary electrophoresis (CE). Chromatographic separation of samples increases the sensitivity of MS detection (by minimizing ion suppression effects associated with complex mixtures and allowing greater sample loading) and provides orthogonal information (retention time prediction) that allows metabolite identification.[2,31,47,62,82] The capabilities of GC-MS and LC-MS techniques have advanced enormously in recent years, with the use of high-resolution, accurate-mass MS instruments, such as the orbitrap MS and Fourier-transform ion cyclotron resonance MS (FT-ICR-MS). New-generation MS instruments that allow post-chromatographic separation of analytes by ion mobility instruments have the capacity to increase metabolite coverage, by allowing separation of metabolite isomers with the same mass and providing information on the shape (collision cross-section) of molecules that can be used for metabolite identification.

Given that no single platform offers complete coverage of either polar or apolar metabolites, the use of multiple complementary analytical platforms is important in initial exploratory studies on new biological systems. On the other hand, targeted metabolomic analyses, which focus on accurate quantification of a smaller number of metabolites, might be appropriate in biomarker studies. Both untargeted and targeted metabolomic approaches require the use of univariate or multivariate pattern-recognition techniques to identify differences between samples and generate new testable hypotheses.[31,78,85] Other exciting opportunities in metabolomics include the use of imaging modalities (such as matrix-assisted laser desorption/ionization (MALDI) and desorption electrospray ionization (DESI) imaging–MS to detect spatial changes in metabolite levels within different tissue types.

Despite its enormous potential, metabolomics has been relatively underutilized in veterinary medicine. Our aim is to comprehensively review, tabulate, and analyze the existing peer-reviewed literature on the use of metabolomics in spontaneous veterinary diseases.

Methods

Search strategy

We searched the public databases PubMed, Web of Science, and CAB using the following search terms:

1. Metabolomic* or metabonomic* or metabolome or metabolic profil* or metabolomic fingerprint* or metabolite*
2. Veterinary* or veterinary medicine/science or livestock or small/large animal
3. Dog* or bitch* or canine* or canid* or canis

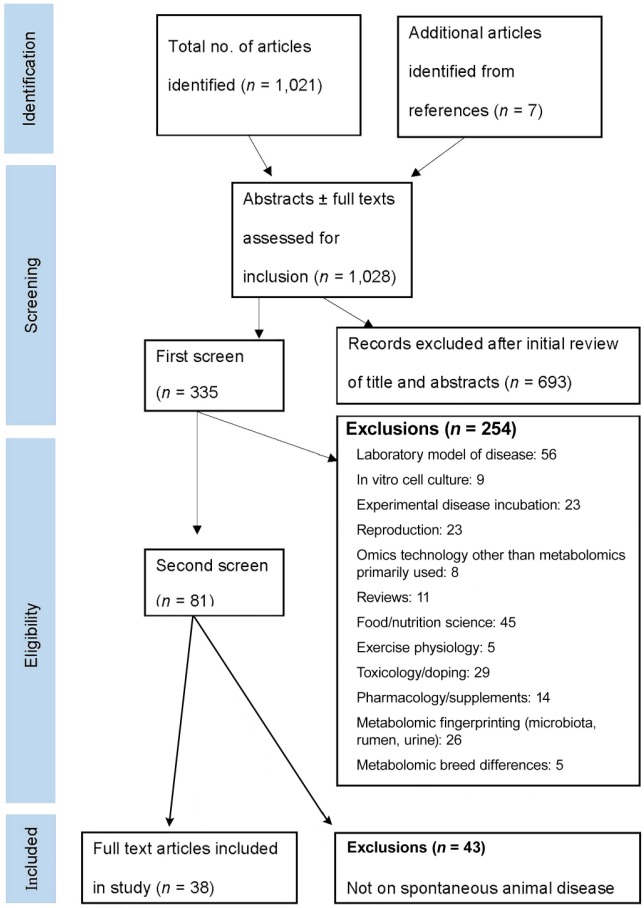

Figure 2. Search and selection strategy. See Table 1 footnotes for definitions of abbreviations.

4. Cat* or feline* or felid*
5. Cow* or cattle or bovine or bovid*
6. Sheep* or ovine or small ruminant*
7. Horse* or equine or equid* or racehorse*
8. Pig* or piglet* or porcine or swine
9. and the combinations (1 and 2) or (1 and 3) or (1 and 4) or (1 and 5) or (1 and 6) or (1 and 7) or (1 and 8)

The use of the asterisk wildcard character allows searching for all possible suffixes. "Or" is used inclusively to search alternative terms (search results contain one or multiple phrases). The literature search (title and abstract) was conducted February 5, 2019 (Fig. 2). The titles, abstracts, or full texts were assessed for eligibility; selected articles were screened, and those that did not meet the inclusion criteria were eliminated. References of pertinent studies were also searched to identify articles for review.

Screening process and data extraction

Studies were eligible for inclusion if they were original, peer-reviewed research articles published in the English language, and if the primary aim was to apply metabolomics to investigate spontaneous animal disease. Exclusion criteria included experimentally induced disease, in vitro cell culture studies, laboratory models of disease, and studies that focused predominantly on an omics field other than metabolomics. Given practical constraints and brevity, we did not include studies on thermal stress, toxicology, or food or nutrition science in our review. Full texts of the relevant studies were retrieved, and data on species, diseases, metabolomic techniques, and results were extracted.

Results and discussion

Thirty-eight studies on metabolomics in veterinary medicine met the inclusion criteria (Table 1). The selected studies were published in 2005–2018. Eighty-eight studies were excluded because the researchers induced disease experimentally or used laboratory animals or cell culture lines to model disease. Forty-five were eliminated because they primarily focused on food or nutrition science; 29 were removed because they focused on toxicology or doping.

Study characteristics

Regarding the technique used, over half of the metabolomics studies (22 of 38) used MS as the sole analytical platform. Two additional studies used FTICR-MS; only one used both NMR and MS platforms.

Regarding the species studied, 13 of the 38 studies were on dogs, 5 on horses, 12 on cows, 3 on small ruminants, 3 on fish, and 1 on birds. Interestingly, only one study focused on feline disease, suggesting that cats are grossly underrepresented in metabolomics studies despite representing a substantial proportion of veterinary patients. No swine studies met our inclusion criteria (Suppl. Table 1).

Regarding the main system investigated, diseases of the digestive system (11 of 38) made up the largest proportion of researched conditions. The second most commonly studied system was the genitourinary or reproductive system, with 7 studies. The remainder of the studies were on systemic disease (n = 3), diseases of neuromuscular or central nervous system (n = 3), the musculoskeletal system (n = 3), the respiratory system (n = 2), the lymphatic system (n = 2), and the cardiovascular system (n = 1). The remaining 6 studies were classified as "other," and included conditions such as canine anxiety, canine diabetes mellitus, bovine milk fever, and bovine ketosis.

Inflammation (including infectious and non-infectious subcategories) represented the largest proportion of disease processes, with 15 of 38 studies; 7 studies concerned neoplasia, 2 vascular diseases, and 3 degenerative conditions. The remainder of the diseases were classified as "other," and were subdivided into metabolic conditions (n = 8) and idiopathic (n = 3).

Table 1. Thirty-eight metabolomic studies on spontaneous veterinary diseases included in our review.

Species	Disease	Sample	Technique	No. of animals	Results	Conclusion	Reference
Canine	GRMD	Skeletal muscle	GC-MS	6 affected, 4 controls	8 altered metabolites in GRMD (decreased stearamide, carnosine, fumaric acid, lactamide, myoinositol-2-phosphate; increased oleic acid, Glu, Pro). Krebs cycle intermediates (malic acid, fumaric acid, citric acid, succinic acid) decreased in GRMD.	Elevated oleic acid in GRMD muscle suggests altered lipid metabolism genes. Elevated L-Arg may serve as GRMD biomarker.	Abdullah et al.[1]
Canine	Epilepsy	CSF	GC-MS	16 idiopathic epilepsy, 19 symptomatic epilepsy, 18 controls	20 of 60 identified metabolites differed among groups. Glu increased in idiopathic epilepsy; ascorbic acid changed in both forms of epilepsy.	Metabolomic CSF profiles of idiopathic and symptomatic epilepsy differ. CSF Glu and ascorbic acid may aid in diagnosis.	Hasegawa et al.[24]
Canine	GM	Serum, bile	GC-MS UPLC-MS	10 affected, 10 controls	GM dogs had decreased serum AMP and fewer metabolites that stimulate biliary ductal fluid secretion (adenosine, cAMP). Increased lathosterol and 7α-hydroxycholesterol, suggesting increased cholesterol synthesis and diversion to bile acid formation.	GM dogs have abnormal regulation of protein and amino acid metabolism. Adenosine, cAMP, taurolithocholic acid, and taurocholic acid are potential GM biomarkers.	Gookin et al.[20]
Canine	Acute diarrhea	Serum, urine, feces	GC-MS UPLC-MS HPLC-MS	13 affected, 13 controls	Diseased dogs exhibited: –decreased fecal *Faecalibacterium* spp. and propionic acid. –decreased kynurenic acid in serum and decreased 2-methyl-1H-indole and 5-methoxy-1H-indole-3-carbaldehyde in urine.	Fecal dysbiosis in acute diarrhea is associated with altered systemic metabolic states.	Guard et al.[22]
Canine	IBD	Feces, serum	GC-MS	12 affected, 10 controls	IBD dogs had: –lower bacterial diversity and distinct microbial communities. –increased serum 3-hydroxybutyrate, hexuronic acid, ribose, gluconic acid lactone.	Alterations in microbiota and serum metabolite profiles persist in IBD dogs despite medical therapy. Oxidative stress implicated in IBD.	Minamoto et al.[44]
Canine	Hepatopathies	Plasma	LC-MS	9 PVA, 6 acquired hepatopathy, 10 controls	Dogs with congenital PVAs had significant disturbances in plasma bile acid and phospholipid profiles.	Metabolomics showed clear differences between groups, not observed with traditional lab parameters. Metabolomics may improve understanding of pathogenesis and has potential as a diagnostic tool.	Whitfield et al.[76]

(continued)

Table 1. (continued)

Species	Disease	Sample	Technique	No. of animals	Results	Conclusion	Reference
Canine	DM	Serum	LC-MS	6 affected, 6 controls	DM dogs showed: –upregulation of glycolysis/ gluconeogenesis intermediates. –downregulation of Trp metabolism metabolites. –decreased bile acids and AAs, except Val, which was elevated in DM.	Differences in metabolomic profiles in DM were similar to those reported in human T1DM (e.g., alterations in glycolysis/ gluconeogenesis metabolites, bile acids, elevated branch-chain AA). Animal models can help investigate human disease.	O'Kell et al.[51]
Canine	DM	Serum	UHPLC-HRMS	6 affected, 6 controls	Downregulation of AAs, LL-2,6-diaminoheptanedioate, and multiple metabolites involved in Trp metabolism (anthranilate, kynurenine, 5-hydroxyindoleacetic acid) in DM. Citramalate upregulated in DM.	Metabolomic profiles differed between DM and healthy dogs. Individual metabolites may be used as DM biomarkers.	O'Kell et al.[50]
Canine	Lymphoma	Serum	GC-MS	21 affected, 13 controls	Dogs with lymphoma had higher levels of 15 metabolites, and lower levels of inositol.	Metabolomics may identify potential biomarkers and aid in diagnosis of canine lymphoma.	Tamai et al.[68]
Canine	Malignant oral melanoma	Plasma	GC-MS	32 affected, 9 controls	12 metabolites increased in melanoma plasma (including citric acid, lactic acid, oleic acid, linoleic acid, palmitoleic acid, octadecenoic acid, glycerol).	Metabolic profile of canine malignant melanoma differs from healthy dogs; metabolomics may identify potential melanoma biomarkers. Energy metabolism elevated in melanoma (exemplifying the Warburg effect).	Kawabe et al.[33]
Canine	TCC	Urine	^{1}H-NMR	40 affected, 42 controls	6 metabolites significantly differed in dogs with bladder TCC.	Metabolomics showed good distinction between groups. Urine metabolic profiling may aid in early detection of bladder cancer and TCC recurrence post-treatment. Canine TCC is a good model of human bladder cancer.	Zhang et al.[85]
Canine	DMVD	Serum	GC-MS LC-MS	18 affected, 11 controls	54 metabolites differed significantly between DMVD and controls (13 of which were previously unknown). Metabolite profiles suggest alterations in fat and Glc energy metabolism and oxidative stress in DMVD.	Identified alterations may benefit from nutritional or medical management. Multi-omics approaches are of growing importance in veterinary science.	Li et al.[41]
Canine	Anxiety, fear	Whole blood	LC-MS	10 affected, 10 controls	Alterations in 13 metabolites (hypoxanthine, indoxyl sulfate, several phospholipids) between groups. Findings suggest oxidative stress and altered Trp and lipid metabolism in fearful dogs.	Significant metabolomic alterations identified, some of which may be relevant to anxiety in other species. Non-targeted metabolomics may aid in identifying biomarkers and pathways of canine anxiety.	Puurunen et al.[56]

(continued)

Table 1. (continued)

Species	Disease	Sample	Technique	No. of animals	Results	Conclusion	Reference
Feline	FORL	Saliva	^{1}H-NMR LC-MS	11 affected, 10 controls	Increased acetate, lactate, propionate, isovalerate, tryptamine, Phe, suggesting altered microflora in FORL. The PLS-DA model predicted FORL cats with >60% accuracy.	Metabolic differences in saliva between groups. Salivary metabolic profiles may be useful in developing a rapid, non-invasive method of FORL diagnosis.	Ramadan et al.[57]
Equine	Septic joint disease	Synovial fluid	^{1}H-NMR	7 septic samples, 10 non-septic samples	Increased acetate, Ala, citrate, creatine phosphate, creatinine, Glc, glutamate, Gln, Gly, Phe, pyruvate, Val in non-septic group. Glycylproline higher in sepsis.	Synovial metabolite panels can distinguish septic and non-septic equine synovial fluid, with Glc the principal discriminator.	Anderson et al.[4]
Equine	OC	Synovial fluid	NMR	5 affected foals, 5 controls	OC samples had reduced Glc, pyruvate, lactate; increased ketone bodies (hydroxybutyrate). Alterations suggest reduced anaerobic glycolytic rate and use of fatty acids as an energy source.	Metabolomics data can help refine equine OC definition, elucidate the molecular mechanisms, and improve diagnosis and treatment for horses and other species.	Desjardin et al.[17]
Equine	OA	Synovial fluid	^{1}H-NMR	25 affected samples, 8 control samples	Increased lactate, Ala, acetate, N-acetylglucosamine, pyruvate, citrate, creatine/creatinine, glycerol, HDL, choline, alpha-Glc in OA samples.	The variations observed in OA are similar to data of other studies; metabolomics may be useful in OA research and treatment of athletic horses.	Lacitignola et al.[39]
Equine	Equine asthma	TW, EBC	^{1}H-NMR	6 affected, 6 controls	10 TW metabolites differed between groups. Asthmatic TW had elevated histamine and oxidant agents; decreased ascorbate, methylamine, dimethylamine, O-phosphocholine.	Results suggest oxidative stress involved in pathogenesis. Metabolomic analysis of TW and EBC may serve as diagnostic tools.	Bazzano et al.[9]
Equine	EMS	Serum	UPLC-MS GC-MS	10 affected, 10 controls	55 metabolites differed between insulin dysregulated and control. 91 metabolites differed between obese and control. 136 metabolites differed between laminitis group and control.	Metabolomics may have diagnostic utility for early disease detection and may expand understanding of pathophysiology.	Jacob et al.[28]
Bovine	Neonatal sepsis	Plasma	NMR	20 affected, 10 controls	Increases in plasma AAs and creatinine in early-onset sepsis indicating response to metabolic deficits. Increased ketone bodies in plasma suggests compensatory response to reduced ATP.	Metabolomics is an excellent tool for fast identification of sepsis and quantification of potential biomarkers.	Basoglu et al.[8]

(continued)

Table 1. (continued)

Species	Disease	Sample	Technique	No. of animals	Results	Conclusion	Reference
Bovine	Neonatal diarrhea and sepsis	Plasma	^{1}H-NMR	4 affected, 11 controls	Decreased lipid soluble metabolites (sphingomyelin, fatty acids) in disease. Altered water-soluble metabolites (increased niacinamide, choline, phosphocholine, 2-methylglutarate, isopropanol; decreased formate, Lys-Arg, acetate, creatine) in disease.	Metabolomics differentiated diarrhea-induced sepsis from controls. Metabolites identified and quantified may be new potential biomarkers for SIRS in calf sepsis.	Basoglu et al.[7]
Bovine	HL	Serum	MS	22 affected, 6 controls	29 metabolites (AAs, phosphatidylcholines, sphingomyelins) differed between control dairy cows and those with different stages of HL.	Metabolomic profiles distinguish HL from other peripartum disorders. Metabolomics is a promising tool for HL diagnosis, pathogenesis, and prevention.	Imhasly et al.[27]
Bovine	Milk fever	Serum	^{1}H-NMR	8 affected, 24 controls	9 metabolites differed between groups (decreased Glc, Ala, glycerol, phosphocreatine, gamma-aminobutyrate; increased b-hydroxybutyrate, acetone, pyruvate, Lys).	Metabolite changes reflect negative energy balance and fat mobilization, suggesting altered energy metabolism in disease. ^{1}H-NMR can provide insight to disease pathogenesis and biomarkers.	Tamai et al.[67]
Bovine	Ketosis	Plasma	^{1}H-NMR	20 K1, 20 K2, 10 controls	7 different metabolites between K2 and C, 19 different metabolites between K1 and C, and 24 different metabolites between K1 and K2. OPLS-DA was more effective than PCA at distinguishing among the 3 groups.	Metabolomics can distinguish differential metabolites among groups, thereby providing information on pathogenesis, early diagnosis, and prevention of K1 and K2 in dairy cows.	Xu et al.[80]
Bovine	Ketosis	Plasma	GC-MS	22 CK, 32 SK, 22 controls	30, 32, and 13 metabolites showed statistically significant differences between SK and NC, CK and NC, and CK and SK, respectively. Results suggests disrupted metabolic pathways in ketosis (fatty acid and AA metabolism, glycolysis, gluconeogenesis, and pentose phosphate pathway). Multiple potential biomarkers identified.	Metabolite differences between groups identified and may have utility in diagnosis, prognosis, and prevention of ketosis. Potential ketosis biomarkers described.	Zhang et al.[83]
Bovine	Periparturient disease (metritis, mastitis, laminitis)	Plasma	MS	6 affected, 6 controls	3 metabolites elevated in diseased cows 4 wk before parturition; 2 metabolites can discriminate diseased cows 1 wk before parturition. A 3-metabolite plasma biomarker profile could predict periparturient diseases up to 4 wk before clinical signs.	Periparturient diseases can be predicted in dairy cattle before their development using a multi-metabolite biomarker model.	Hailemariam et al.[23]

(continued)

Table 1. (continued)

Species	Disease	Sample	Technique	No. of animals	Results	Conclusion	Reference
Bovine	Metritis	Urine	NMR	6 affected, 6 controls	30 altered metabolites in pre-metritic cows 8 wk before parturition; 28 of which increased in urine. 34 metabolites altered 4 wk before parturition. At the week of metritis diagnosis, 20 metabolites were altered.	Metabolic fingerprints in urine of pre-metritic and metritic cows suggest excretion of AAs, tricarboxylic acid cycle metabolites, monosaccharides. Galactose, Leu, Lys pantothenate at 8 wk before parturition might serve as predictive biomarkers.	Dervishi et al.[15]
Bovine	RFM	Serum	LC-MS	6 affected, 20 controls	128 metabolites identified and quantified at different stages pre- and postpartum in both groups. Major metabolite fingerprint alterations detected in pre-RFM cows 8 wk before and after calving. Decreased LPC, Trp, and higher kynurenine prepartum and the week of occurrence of RFM suggest inflammation.	RFM dairy cows is preceded by alterations in multiple metabolites starting from 8 wk before parturition. Identified serum biomarkers related to nonspecific inflammation.	Dervishi et al.[16]
Bovine	SCM	Serum	GC-MS	6 affected, 20 controls	13 metabolites altered in SCM cows 8 wk prepartum; 17 metabolites altered the week of SCM diagnosis; 10 and 11 metabolites altered in SCM 4- and 8-wk postpartum, respectively. Val, Ser, Tyr, Phe are good predictors of SCM at 8- and 4-wk pre-calving.	SCM is preceded and followed by alteration in AA metabolism. Val, Ile, Ser, Pro may be SCM biomarkers in early lactation and at 4–8 wk after parturition.	Dervishi et al.[14]
Bovine	Mastitis	Milk	UPLC MS	20 CM, 20 SCM, 20 controls	Glc, 4-hydroxyphenyllactate, l-carnitine, sn-glycero-3-phosphocholine, citrate, hippurate decreased in CM milk. Benzoic acid, l-carnitine, cis-aconitate decreased in SCM milk. Both CM and SCM milk had elevated Arg and Leu-Leu, and decreased d-glycerol-1-phosphate.	Significant variations detected between groups. Metabolomics can help better understand the pathobiology of mastitis, but clinical validation needed before field application.	Xi et al.[79]
Bovine	Mastitis	Serum	UPLC-MS	8 affected, 9 controls	CPM serum had elevated 3′-sialyllactose and inflammatory markers (SAA, visfatin). 7 metabolites could classify cows for their future CM status at 21 and 14 d before calving.	Metabolic phenotypes suggest elevated protein and lipid metabolism and inflammation precede CM in prepartum transition dairy cows. Identified metabolites may aid in CM diagnostics, prevention strategies, and early treatment and thereby improve cow health and welfare.	Zandkarimi et al.[81]
Ovine **Caprine**	LR	Brain biopsies	^{1}H-NMR	13 affected (8 sheep, 5 goats), 12 controls	LR brainstem biopsies had decreased NAA, N-acetylaspartylglutamate, choline, myo-inositol, scyllo-inositol; increased Gly, phosphocholine, taurine, lactate.	Metabolic profiles of brainstem biopsies were altered in LR.	Precht et al.[55]

(continued)

Table 1. (continued)

Species	Disease	Sample	Technique	No. of animals	Results	Conclusion	Reference
Ovine, Caprine	CLA	Serum	NMR	5 sheep, 10 goats treated with AgNP-based cream; 4 sheep, 10 goats treated with iodine	All animals showed stable serum metabolomes when iodine or AgNP-based cream effects were compared. Wound healing was faster with AgNP-based cream treatment compared with the iodine treatment.	The PLS-DA did not show separation of the groups, suggesting both treatments affected metabolism similarly when serum metabolites compared.	Stanisic et al.[64]
Ovine	CLA	Serum	^{1}H-NMR 2D NMR	33 affected, 26 controls	20 metabolites were altered between groups; 9 metabolites were found only in the healthy sample group and 5 metabolites in CLA.	Exclusive *Corynebacterium pseudotuberculosis* metabolites can be observed with NMR-based metabolomics. Data may help develop noninvasive diagnostic method.	Pontes et al.[54]
Flatfish	HCA	Liver tissue	FTICR-MS LC-MS	21 HCA fish, 12 controls	Betaine and choline decreased in HCA compared with normal fish liver tissue distal to tumors; changes suggestive of alteration in energy metabolism and the one-carbon cycle.	Metabolomics, combined with DNA methylation and transcriptomics, allowed better understanding of HCA in fish	Mirbahai et al.[45]
Flatfish	HCA	Liver tissue	^{1}H-NMR	10 diseased, 10 controls	Negative correlations observed between Ala-acetate and between Pro-acetate in HCA only, suggesting Ala and Pro are utilized as alternative energy sources in flatfish liver tumors.	Metabolomes of healthy and HCA livers differed, which underscores the different metabolic demands between tissue types.	Southam et al.[63]
Flatfish	HCA	Liver tissue	FTICR MS	9 affected, 9 controls	Metabolome of HCA tissue differs from non-tumor liver; however, molecular differences were considerably greater between fish than between HCA and controls.	Multi-omics approaches can be used to discriminate between tumorous and non-tumorous liver.	Stentiford et al.[65]
Avian (falcon)	Aspergillosis	Plasma	^{1}H-NMR	17 affected, 12 controls	In disease, 3-hydroxybutyrate greatly increased; Leu, Ile, Phe moderately increased.	Clear metabolic differences detected between groups. Metabolomics is a powerful diagnostic tool for aspergillosis.	Pappalardo et al.[52]

Diseases/groups: C = controls; CK = clinical ketosis; CLA = caseous lymphadenitis; CM = clinical mastitis; CPM = clinical post-calving mastitis; DM = diabetes mellitus; DMVD = degenerative mitral valve disease; EMS = equine metabolic syndrome; FORL = feline odontoclastic resorptive lesions, GRMD = Golden Retriever muscular dystrophy; GM = gallbladder mucocele; HCA = hepatocellular adenoma; HL = hepatic lipidosis; IBD = inflammatory bowel disease; K1 = type I ketosis; K2 = type II ketosis; LR = listerial rhombencephalitis; OA = osteoarthritis; OC = osteochondrosis; PVA = portosystemic vascular anomalies; RFM = retained fetal membranes; SCM = subclinical mastitis; SK = subclinical ketosis; TCC = transitional cell carcinoma.
Samples: ebc = exhaled breath condensates; TW = tracheal wash.
Techniques: ^{1}H-NMR = proton nuclear magnetic resonance; 2D NMR = two-dimensional nuclear magnetic resonance; FTICR = Fourier-transform ion cyclotron resonance; GC-MS = gas chromatography–mass spectrometry, HPLC-MS = high-performance liquid chromatography–mass spectrometry; LC-MS = liquid chromatography–mass spectrometry; MS = mass spectrometry; NMR = nuclear magnetic resonance; OPLS-DA = orthogonal projections to latent structures discriminant analysis; PCA = principal component analysis; PLS-DA = partial least squares discriminant analysis; UHPLC–HRMS = ultra-high performance liquid chromatography–high-resolution mass spectrometry; UPLC-MS = ultra-performance liquid chromatography–mass spectrometry.
Other: AA= amino acid; AgNP = silver nanoparticle; Ala = alanine; Arg = arginine; CSF = cerebrospinal fluid; Glc = glucose; Gln = glutamine; Glu = glutamic acid; Gly = glycine; Ile = isoleucine; Leu = leucine; LPC = lysophosphatidylcholine; Lys = lysine; NAA = N-acetylaspartate; Phe = phenylalanine; Pro = proline; SAA = serum amyloid A; Ser = serine; Trp = tryptophan; Tyr = tyrosine; Val = valine.

All bovine studies were related to reproductive diseases of economic importance, including metritis, retained fetal membranes, mastitis, and milk fever. This may reflect the growing interest in the use of multi-omics techniques for production improvement, a trend that has been demonstrated previously in agricultural sciences.

During our search, we found numerous studies that did not meet the inclusion criteria but are nonetheless relevant to veterinary science. These studies were outside the scope of our review. Evaluation of the quality of published papers was also beyond the scope of our study.

Principal findings

All studies in our review detected statistically significant differences in the metabolome of diseased and non-diseased states, suggesting that nearly all spontaneous diseases of veterinary interest are characterized by altered host cellular and/or microbial metabolism. In fact, most studies identified 10 or more metabolite differences in the diseased subjects compared with controls. Seventeen studies identified specific metabolites that could serve as biomarkers of the disease of interest in a clinical setting. A *biomarker* is a measurable biochemical indicator of a biological state, including normal or pathologic processes;[46,71] in addition to their diagnostic utility, biomarkers can also help monitor a patient's response to treatment. As an example, a 2018 study used a targeted metabolomics approach to evaluate the pathogenesis of retained fetal membranes in dairy cows, and to identify potential biomarkers that may serve as early predictors of disease.[16] Multiple metabolite alterations were identified as early as 8 weeks prepartum; however, many of the metabolites reflected the presence of inflammation and may not be specific to retained fetal membranes. This highlights the importance of establishing whether changes in the metabolome are specific to disease when evaluating the utility of metabolomics in biomarker discovery.[34] Other challenges in developing biomarkers in veterinary medicine include validation and qualification of biomarkers,[46] which no study in our review achieved.

Our study also reveals that metabolomics in veterinary medical research can complement our understanding of human disease. As an example, a 2012 study on canine transitional cell carcinoma found that affected dogs had increased levels of citrate and beta-hydroxybutyrate in their urine.[85] These aforementioned metabolites are similarly elevated in the serum of humans with esophageal adenocarcinomas, suggesting changes in Krebs cycle activity in epithelial malignancies,[84,85] regardless of the host species. Similarly, a 2017 study identified similar serum metabolites in canine diabetes mellitus and human type I diabetes, including changes in glycolytic intermediates and elevated levels of branch-chain amino acids.[51]

Reflecting the increasingly recognized importance of the microbiome, multiple studies explored the relationship between host health and the gastrointestinal microbiota.[22,44] As an example, dogs with acute diarrhea were found to have decreased fecal concentrations of *Faecalibacterium* spp. and propionic acid, a short-chain fatty acid (SCFA).[22] Although host–microbiome interactions are complex and dynamic, this finding suggests that dysbiosis in acute diarrhea may have a direct impact on SCFA concentrations.[22] Additionally, as circulating metabolites may be derived from microbes rather than host cells,[25,48] observed metabolite alterations may reflect microbiome changes rather than host cellular changes in disease states. Further research on the gut microbial–host co-metabolism is needed to improve our understanding of disease pathogenesis and potential treatments.

Many included studies did not acknowledge or control for concurrent processes that may arise in disease, such as inappetence or dehydration. Therefore, it is challenging to determine whether the observed metabolite alterations are a result of the disease or the result of a concurrent process. Given that diseased animals often exhibit reduced feed intake and lethargy, researchers should consider potential confounding effects when evaluating the strength of association between metabolite changes and disease.

Limitations of metabolomics

Although metabolomic techniques have evolved since its inception, there are a number of limitations that hinder its widespread use. At present, most metabolomic studies only identify a minority of metabolites in biological samples, reflecting the complexity of sample analysis, the presence of multiple adducts and isotopes for each species, and the difficulty of validating 100s of metabolites with suitable standards. In addition, some classes of metabolites are either difficult to detect using current instrumentation or are present below the level of detection. Incomplete coverage of key metabolic pathways can complicate the interpretation of data. Although the identification of unknown or poorly defined metabolites remains one of the biggest challenges for metabolomics, the detection of new or unanticipated metabolites also presents new opportunities for understanding disease processes and detecting new disease biomarkers.[60,73]

Another challenge in metabolomics is determining the significance or role of identified metabolites. Non-targeted metabolomic analyses generate enormous data sets that may contain vast amounts of clinically irrelevant information. The complexity of the metabolome and limitations in computational software technologies and algorithms make it challenging to extract relevant data.[19] Transforming these data into valid interpretations and conclusions requires an in-depth understanding of metabolic pathways and the interconnectivity of metabolites and biological systems.[30] In many cases, it is difficult to conclude how a change in metabolite steady-state levels translates to changes in metabolic fluxes through one or more associated pathways, although this is increasingly being addressed by coupling metabolomic

approaches with stable isotope labeling. Another point to consider is that some metabolites are only biologically significant in the presence of other metabolite(s), which makes pattern-recognition analyses a particularly important component of metabolomics.

Additional challenges that need to be addressed are the development and optimization of sample collection and storage protocols for veterinary studies. Finally, metabolomic data alone is often insufficient in gaining a global understanding of physiologic processes; therefore, integrating multiple omics technologies (such as genomics, proteomics, or transcriptomics) may provide a more holistic perspective than any single omics field alone.[10,53]

Conclusion

Our literature search revealed that metabolomics has been applied widely in various animal science disciplines, but relatively few studies focused on spontaneous animal disease. Employing techniques such as NMR spectrometry and MS, metabolomics enables characterization and analysis of numerous metabolites in a biological sample. Metabolomics has immense potential in the study of spontaneous veterinary disease and may facilitate biomarker discovery and improve our knowledge of disease pathogenesis. Other opportunities include tracking response to treatment, pharmaceutical development, and toxicologic studies. Although there are relatively few metabolomics studies to date, we anticipate that many more will be performed in the future.

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ORCID iDs

Helena Tran https://orcid.org/0000-0002-2191-5483

Panayiotis Loukopoulos https://orcid.org/0000-0002-4308-8618

Supplementary material

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