Title: Breath analysis for disease detection in dairy cattle: where are we after three decades of research?

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Timely detection of diseases on dairy farms is critical from a health, welfare, and economic point of view. In practice, diseased cattle are often detected only when the clinical signs are apparent. Exhaled breath is a complex mixture of molecules that reflects the composition of blood volatiles. Breath can be collected non-invasively and frequently, offering an alternative to the stressful and often belated current diagnostic procedures. We performed a systematic literature review on using breath analysis for disease detection in dairy cattle. Our initial search identified 202 records from Web of Science and Scopus. After excluding duplicates and nonrelevant records, 10 papers remained for in-depth review. Our results show that research focused on cows before 2000, however, calves have been studied more frequently since then. Studies on calves focused predominantly on infectious diseases (75.0%), whereas metabolic diseases were in the spotlight of most studies on cows (80.0%). Ketosis was the most studied disease (40.0% of included papers). The median number of farms and animals were 1 and 12, respectively. The gaseous phase of the exhaled breath was analysed in 70.0%, and the exhaled breath condensate in 30.0% of the studies. Each study used a face mask or a hand-held device for sample collection, and most (70.0%) studies targeted or identified specific compounds. All studies concluded that it was possible to distinguish diseased cattle either based on specific biomarkers or by classification models. In conclusion, our knowledge about breath biomarkers of various dairy cattle diseases is still very limited. Opportunities for future research include, i.a. analysis of the alveolar fraction of breath, and conducting larger studies that allow for more robust conclusions. Once the patterns of the biomarkers are better understood via controlled sampling, we can make the next step forward to automated detection of diseased cattle using breath.