Final report

Agscent Cow Breath Sampling: pregnancy diagnosis proof of concept

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Abstract

This project aimed to build on the early stage learnings achieved through the Agscent cow breath sampling and testing prototype development (P.PSH.1166) and proof of concept demonstration.

This project sought to validate these findings and develop a pregnancy screening device which would include a composite sensor, with increased sensitivity to achieve day 40 pregnancy diagnosis using nano-sensors which analyse breath bio markers, making remote detection easier. The project also aimed to begin limited commercialisation of the technology. Detection of pregnancy involved three steps:

1. Collection of breath from a cow
2. Processing of breath through both GC-MS analysis and an e-nose device
3. Running algorithms which modelled pregnancy

During the funding project period, a hand held device was developed to ensure the collection of breath of cows was distinct from ambient air under a full range of farming weather and yard conditions. Further, additional funds were raised from investors to further refine our understanding of bio markers relevant to pregnancy as the new device allowed us to obtain cleaner biological breath samples in all weather conditions. The e-nose sensors we have used from the beginning continued to model pregnancy, however they were not able to achieve the sensitivity and specificity required by MLA. Further, the manufacturers of these sensors were significantly affected by Covid-19 making their use in our device unachievable.

We can report, however, that the bio markers analysis can achieve the required sensitivity and specificity, however, this can only be completed using equipment not yet developed for field use. We are now focused on trialing new sensors which would operate in our device under an evaluation license with both NASA and a Canadian company who are modifying nano-sensors designed as part of Covid research for our use. We will continue to monitor breathomics developments in human medicine science and apply them to our breath collection and diagnostic methods.

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1. Background

Primary producers make money every time they sell an animal. Understanding when a cow is pregnant is critical to livestock production and producer financial viability. Our research and product development has identified a process using a unique (and novel) breath collection device which will disrupt the way animals are pregnancy tested in the future. We are focused on the pursuit of 'no pain, no stress' livestock management and believe by developing a device which can collect breath and analyse it for pregnancy, we are creating a gateway technology which is non-invasive, cost effective and accurate for many livestock health needs.

We are the first in the world to identify biomarkers which distinguish pregnant from not pregnant cows. We are also adapting e-nose sensors from medical and industrial use for application in a crush side handheld unit which can collect cow breath (as opposed to ambient air) in all weather conditions.

2. Objectives

The principal project objective was to develop and refine, to a point of commercialisation, a non-invasive point of need (field) device capable of early detection of pregnancy in cattle as an alternative to palpation or ultrasound. Our objective include a report to MLA detailing the completion of of validation testing in at least 100 cows with achievement criteria being:

a) accuracy: <3% false positive [specificity], and <5% false negative [sensitivity]) for pregnancy testing at 40 and 60 days in cows with known breeding/ insemination dates, using rectal palpation and ultrasound for comparison, and eventual calving results for confirmation. Cows tested pregnant, but not delivering a calf at term, will be appropriately inspected for a possible cause of foetal loss, and
b) Operational speed: min 60 cows per hour.

3. Methodology

We proposed to achieve the above objectives by:

1. Validating the initial findings in partnership with the Department of Primary Industries (DPI) by using a herd under their management and on a number of secondary herds within Carwoola Pastoral Company – pregnancy at 60 days +
2. Testing and further refining the design of a composite sensor (including a more sensitive digital nose) which recognises the pattern of volatile compounds detecting pregnancy at 40 and 60 days.
3. Testing other light sensing technologies which may be able to be used to respond visually to the compounds, making remote detection possible and enhance the ease of use of the device.
4. Undertaking research to identifying the exact specification of each volatile compound and its pattern which detect pregnancy for inclusion in the patent.
5. Developing the software to enable farm management software or farm equipment (such as Te Pari's auto drafter) to access the results.
6. Producing a saleable instrument.

Research Program

The research program has included sampling over 1400 cows from 9 herds between late 2018 and March 2021. The herds include animals from Garangula (Romani Pastoral Company), Clouds and Carwoola (Carwoola Pastoral Company), Yurramie Dairy and a TOCAL dairy herd under the supervision of the DPI.

Breath samples have been taken using four versions of the breath sampler with each iteration requiring significant materials testing and design/prototyping activities. In addition, each of these samples have been analysed using GC-MS equipment and personnel from either Sydney University or CSIRO and analysis by with the e-nose device.

In total we have sampled on more than 35 days and the data has been analysed using a variety of statistical methods, principally PCA (Principal Component Analysis) and PLS-DA (Partial Least Square – discriminate analysis). In addition to using Chemanalyst, Brad Swarbrick, of Quality by Design, we engaged Idletechs from Norway to conduct an independent analysis of our data to ensure our findings stood up to international independent scrutiny.

We are preparing academic papers for publication, however due to commercial in confidence requirements, these are being held for a period.

We have submitted a number of patent applications and design registrations to protect our IP as we have progressed. These include:

Patent Application 1:
This relates to the identification of a biomarker or biomarkers in an animal's breath (references the likelihood that this invention will be used with pigs, sheep and other livestock animals). Titled 'Method for determining the pregnancy state of an animal' (2019901686), filed on the 19 May 2019 and updated in May 2020.

Patent Application 2:
This relates to the Agscent Agritech Breath Diagnostic Device, which is used to collect animal breath. Titled 'Biological Sample Capturing Device' patent application no. 2019901085, filed 31 March 2019 and updated in May 2020.

Patent Application 3:
This relates to the Biological Sample Analysis Device with Associated Capturing Device and Associated Software. Patent application no. 2020903361, filed 18 September, 2020

Registered Designs

- Design number 201911826 Breath Sampling Device. Filed 31 March 2019. Registration date 21 May 2019.
- Design number 201911827 Nose Piece for Breath Sampling Device. Filed 31 March 2019. Registration date 24 May 2019.
- European Union Design Registration number 006954650-0001 and 006954650-0002.
- United States Patent Application No. 29/707,648 titled Nose Piece filed September 30, 2019.

Other background IP and unique know how
Proprietary e-nose sensors and analytics, including algorithms arranged to identify volatile substances in a breath sample from a mammal and analyse the breath sample to determine a

medical or physical condition in the mammal such as for example pregnancy and bovine respiratory disease, and ongoing improvements to the algorithms.

Ethics Approvals

This research has been conducted as approved (TRIM 18/362) by the NSW Department of Primary Industries: THE SECRETARY'S ANIMAL CARE AND ETHICS COMMITTEE.

4. Project outcomes

4.1 Validating the initial findings in partnership with the Department of Primary Industries (DPI) by using a herd under their management and on a number of secondary herds within Carwoola Pastoral Company – pregnancy at 60 days +

Blind validation tests were conducted with the DPI which using a very early prototype of the breath collection device with e-nose sensor processing completed on the sample separately. The DPI report identified the significant animal welfare improvement achieved from our approach and noted the ease of use for operators. The DPI identified that operational speed was on average 10 seconds for breath collection and 60 seconds for e-nose processing, however we did not achieve the sensitivity and specificity at that time.

Subsequently have have undertaken significant redesign of the breath collection device (breath diverter) to improve consistency between captured breath samples. This has now been tested on a number of Carwoola Pastoral Company herds in addition to Romani Pastoral Company's Garangula site and a dairy on the South Coast of NSW.

The breath diverter now includes numerous sensors and technologies which open and close when the cows breath is of a Co2 level representative of significant levels of end tidal breath. Our analysis shows that we can consistently differentiate between ambient air and cows breath in rain, dust, smoke, heat, cold and fog.

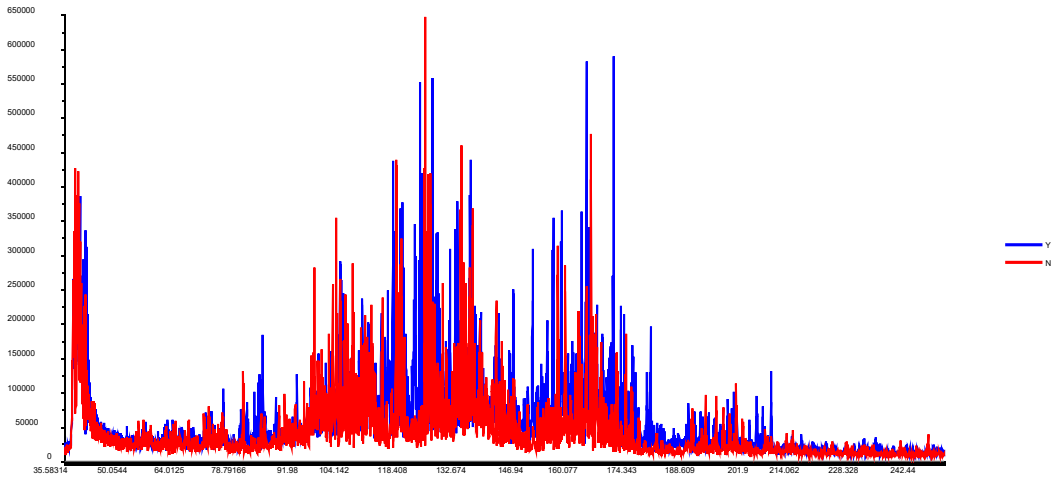
This device, while not including the e-nose sensors which detect pregnancy, allows for the collection of a high value biological sample. It is now being used in the Bovine Respiratory Disease project with MLA.

4.2 Testing other light sensing technologies which may be able to be used to respond visually to the compounds, making remote detection possible and enhance the ease of use of the device.

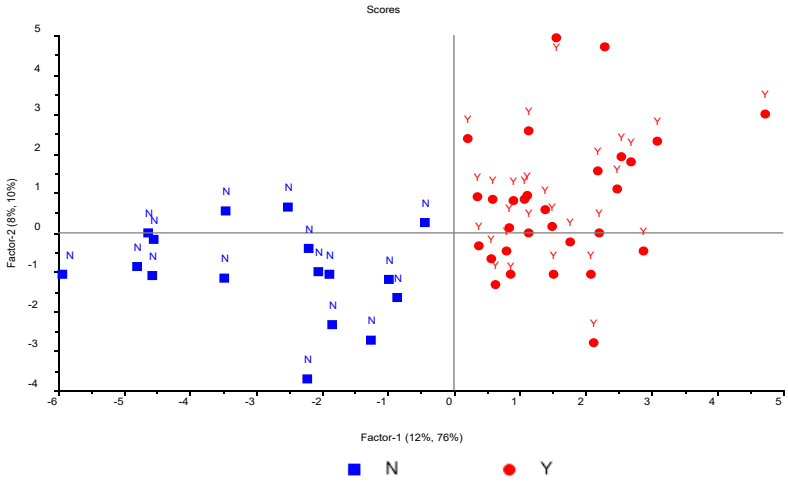
The VOC's we are identifying are not visible under any current light sensing technologies however we are now trialing nano sensor technology from NASA which we hope will meet the required objectives of sensitivity, specificity and operational speed for this project.

4.3 Undertaking research to identifying the exact specification of each volatile compound and its pattern which detect pregnancy for inclusion in the patent.

An initial patent was filed outlining the core VOC's with significant new discoveries being made which will lead to additional patent applications. The use of our new breath diverter device has allowed us to analyse a higher quality breath sample leading to greater insights into VOC's of relevance for pregnancy. This has resulted in distinguishing VOC's from day 20 onwards.



Average spectra for pregnant/empty 20 days



Validated model differentiating pregnant/empty cows at day 40 (100%)

4.4 Developing the software to enable farm management software or farm equipment (such as Te Pari's auto drafter) to access the results.

This has been put on hold until the device is operational in its own right at crush side. Upon reflection, this was not likely to be achievable with the initial funding from MLA.

4.5 Producing a saleable instrument.

This was definitely an overreach for the research proposal as much work still needs to be done to achieve this. In saying that, we now have funding to move us more quickly to this end once the e-nose sensor technology is finalised and validated to the levels of sensitivity and specificity required.

5. Key messages/recommendations

The original MLA financial support has now been leveraged to attract significant additional funds from investors. In addition to this, we have attracted a $600k grant from Accelerating Commercialisation to support the device commercialisation. We have also been awarded a grant from Innovation Connections to support the employment of a Technical Field Officer, with a Bachelor of Animal and Veterinary Bioscience. We have also been able to employ both a young and older person from agriculture into our regional business in addition to technical staff who have enjoyed getting out of the lab and into the yards.

Based on the findings of the research funded by MLA, the following notes some relevant key messages and recommendations.

- We have attracted world-wide interest in supporting us to solve the problem we are trying to overcome. It has become very clear that pregnancy detection using our technology is highly attractive to both customers and funders.. There is a serious market opportunity here and we believe the early support of MLA, has been valuable in providing research support for a very new area of science which has such great potential to transform the way we manage livestock health.
- To be able to measure the volatile organic compounds in breath in such a complex and messy environment has resulted in the invention of a device which can then have multiple sensors incorporated to support various diagnostic scenarios. This became the focus of our tech development during Covid-19 where sensor access and significant barriers to site trials constrained us.
- The original funding proposal included performance criteria which we will continue to work to achieve. We recognise that achieving the sensitivity, specificity and operational speed established for this project by MLA is important to industry. We will continue to pursue achievement of this.
- Covid-19 has provided an opportunity to piggyback on further development in the world of sensors and breath analysis which has grown significantly over the past 12 months. We are actively pursuing these developments and have established trials to test new and emerging sensors.
- The media relating to what we are doing has attracted many in our own industry to offer their herds for testing. We will continue to work with these often very large producers to validate our product as it develops.

- An unexpected finding of our research has allowed us to potentially identify individual cows prior to AI with fertility issues. It appears that there are specific biomarkers identifiable prior to AI which indicate which cows will not become pregnant after AI. We propose to further research these biomarkers further. We believe further research in this area would be significant value to primary producers.
- We have an agreement to assess the effectiveness of a number of NASA sensors which will require additional significant funding should they prove effective in detecting pregnancy or disease. We propose to apply to MLA for further financial support if this occurs within the sensitivity, specificity and operational speed requirements originally established by MLA.
- Animal handling methods and their risk to farm staff has been highlighted during our research. Our hand held device is best used in a crush with a chin lift. We have regularly been faced with farm staff who 'handle' animals in a crush in a way which in itself involves unreasonable personal risk. We are including consideration of animal handling restraints as part of the ongoing research into our product development. Ideally, we will incorporate our sensors into a robotic arm which removes the need for human interaction in order to diagnose a variety of health states.