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Detection of cutaneous myiasis in sheep using an 'electronic nose'

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ABSTRACT

Cutaneous myiasis (flystrike), in Australia caused primarily by *Lucilia cuprina* [Diptera: Calliphoridae], is a debilitating, painful and potentially lethal disease of sheep. Early detection of flystrike is difficult and continual flock surveillance is required to enable timely treatment of struck sheep. Electronic nose technology offers the potential for early and automated detection of flystrike.

An electronic nose consisting of six metal oxide semiconductor sensors and temperature and humidity sensors was used to measure odours collected by dynamic headspace sampling during flystrike development in four experiments and from urine- and faeces-stained fleece in one experiment. Non-linear signal measurement techniques and linear discriminant analysis (LDA) were used to extract signal features and process those features for analysis of categorical separation of odour groups.

The results from LDA indicated that the electronic nose accurately distinguished flystrike odour on days 1, 2 and 3 of development from that of dry wool in all experiments ($P < 0.05$). The electronic nose was also able to discriminate flystrike odour on the day of larval implantation (day 0) in three of the four studies. In the experiment with urine- and faeces-stained wool, these odours were accurately distinguished from both dry wool and flystrike ($P < 0.05$).

This study provides proof-of-concept for the detection of flystrike using electronic nose technology. Practical methods for collection of odour in the field and suitable detection algorithms will be required for development to commercial application.

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1. Introduction

Flystrike (cutaneous myiasis) is a debilitating and potentially lethal disease of sheep that occurs when gravid female flies lay their eggs in moist or stained areas of the fleece, the eggs hatch and the larvae begin to burrow into and feed on body tissues. Flystrike is of major economic impact to sheep production in the southern hemisphere and Britain, inflicts pain and suffering and causes the death of millions of sheep each year (Scholtz et al., 2000; Fraser et al., 2006; Heath and Bishop, 2006; Sackett et al., 2006). During the flystrike season, flocks must be monitored for the occurrence of clinical signs of flystrike. If strikes are not promptly detected and treated, death from strike by *Lucilia cuprina* can result in 6–8 days (Guerrini, 1988). Monitoring consists of regular paddock inspections or mustering sheep for more intensive examinations. This requires a substantial labour input and represents a significant cost for sheep owners. In addition, strikes are difficult to detect in the early stages and can be well advanced before they are found.

Flystrike produces a characteristic foul odour, which attracts flies and stimulates further oviposition (Mackerras and Mackerras, 1944). Sheep dogs that can detect fly struck sheep, presumably from olfactory cues, are well known in the sheep industry. This has led to much interest in the

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potential of electronic nose (e-nose) technology for automated and early detection of flystrike.

Electronic noses attempt to mimic mammalian olfaction systems using an array of sensors that react to volatile chemical compounds (Bourgeois et al., 2003). The sensors produce chemical or physical responses that can be transduced to electrical signals or digital output. The pattern of responses or odour 'fingerprint' obtained from the array can be analysed by pattern recognition algorithms and thus, used to discriminate between a wide range of odours (Sohn et al., in press). This technology has been used widely in various applications such as quality control in the food and beverage industry (Lozano et al., 2008), detection of gas leaks and pollutants (Perera et al., 2006; Sohn et al., 2008) and human (Dragonieri et al., 2009) and animal disease diagnosis (Fend et al., 2005).

Electronic nose technology offers the potential for the early detection of flystrike due to its capability of odour discrimination. In this study, a series of experiments with an electronic nose was conducted to provide "proof-of-concept" for the use of electronic nose technology in the detection of fly strike in sheep.

2. Materials and methods

2.1. Odour induction

Flystrike was induced by a method modified from that of McLeod (1937). For each implant the skin at the implant site was lightly scarified and approximately 400 first instar *L. cuprina* larvae applied under moistened cotton wool plugs held in the wool with binder clips. At 24 h the implants were inspected and if a strike had established, cotton wool pads and clips were removed. Infested sheep were closely monitored throughout each sensing period and following completion of odour sensing on day 3, larvae were manually removed, soiled wool was shorn away and the infested area was treated with spinosad (Extinosad®, Elanco Animal Health, 120 Wharf Road, West Ryde NSW). For urine and faeces odour, 50 ml of sheep urine or sheep faeces mixed in slurry diluted 1:1 with water was applied daily (after the completion of the day's odour sampling) to an approximate area of 50 cm^2 fleece on the back of the test sheep. All experiments were conducted according to ethics committee guidelines under Queensland Primary Industries and Fisheries Animal Ethics Committee approval number SA2006/07/133.

2.2. Electronic nose

The electronic nose was fitted with six metal oxide semiconductor (MOS) sensors chosen on the basis of gas chromatography–mass spectrometry (GC–MS) profiles of odour from flystrike, as well as a temperature and a humidity sensor (Enose Pty Ltd., Sydney, Australia). A data acquisition device (Pico™ Crag Technologies Inc., Garden City, KS) was connected to the sensors to relay the potential changes in sensor responses to a computer for recording and illustration.

The outputs from the sensor array in the electronic nose can be influenced by the changes in the surrounding environmental factors such as temperature, humidity and air velocity. To minimise confounding effects of external influences and improve the signal to noise ratio, the electronic nose was housed in a customised low-pressure (<2 psi) stainless steel chamber. The housing chamber was connected to a sample delivery and purging system (Fig. 1).

A diaphragm vacuum pump (Thomas, Sheboygan, USA) was used to draw odour or air to the electronic nose via odourless sampling tubes (Teflon®, 6.35 mm outside diameter). The air flow was controlled by two solenoid valves mounted in the sample delivery and purging system as shown in Fig. 1. The solenoid valves were automatically switched according to the predetermined sampling

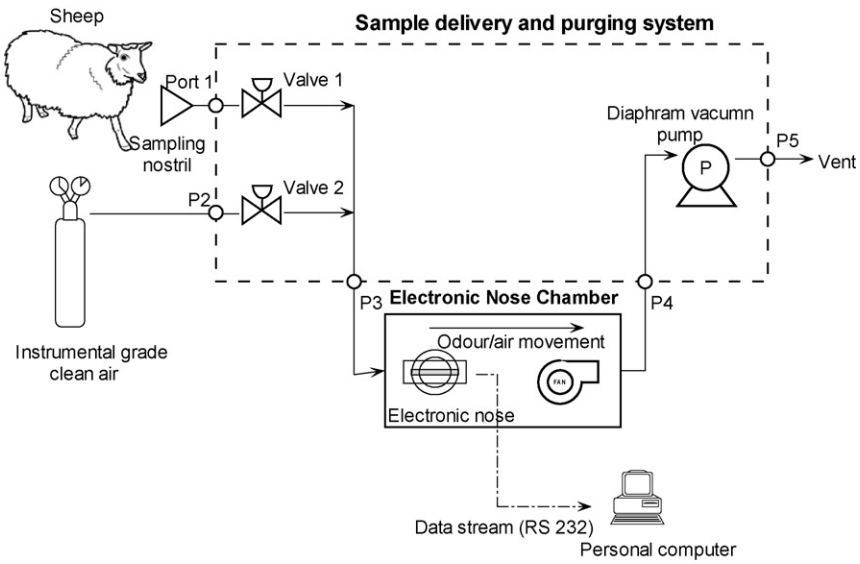

Fig. 1. Schematic diagram of the experimental system, which included the odour or clean air source, the sensor array enclosed in an airtight chamber, an odour delivery system, moisture trap and computer for data logging and recording.

schedule using a Mitsubishi α programmable logic controller (NHP Pty Ltd., Toowoomba, Australia). The air sampling flow rate was controlled using a precise needle valve (Swagelok®, Brisbane, Australia) after calibration using a TSI® 4000 digital flowmeter (Kenelec Scientific Pty Ltd., Mitcham, Vic, Australia). The sample delivery and purging system and the electronic nose chamber were constructed using odourless materials such as SS 316 food-grade stainless steel and Teflon tubes to prevent any possible odour emission. During the sampling phase, the odour samples were collected using a glass funnel with a glass moisture trap built-in. Clean air from an instrument-grade air cylinder (BOC, Brisbane, Australia) served as a reference and was used for purging the sensor array of the electronic nose (*i.e.* returning the signals from the sensor array back to baseline) and sample delivery system.

In experiments 1–3 the sheep were restrained in a small pen and a glass funnel with internal volume of 90 cm^3, resting on the fleece surface was used to directly sample odour from the control, flystrike or stained wool sites. In experiment 4, the sheep was held in a chamber 2 m long, 1.2 m wide and 1.9 m high, and the intake tube and odour collected through a port 0.7 m above the level of the sheep's back.

2.3. Odour collection regime

Odour samples were collected by dynamic headspace sampling at 20 min intervals between 10 am and 4 pm on each test day. Samples were collected on the day prior to odour induction in all experiments and also from additional untreated sheep in experiments 1–3 (Table 1). The sensor array sensitivity was set at low for experiment 1 (setting 1 of 15 levels) and moderate (9 of 15 levels) in experiments 2–4.

The sensor array system was allowed to sample reference air for about an hour prior to acquisition of the first sample in each experiment to stabilise channel signals. In experiments 1 and 2, odours were sampled at 2 L per minute for 1200 s followed by reference air for 1200 s. In experiments 3 and 4, odours were sampled for 60 s or less followed by 1200 s of reference air (Table 1). At the end of each day's odour sampling the sensor array system was flushed with reference air for about an hour to return the sensor responses to baseline and minimise any residual contamination of the system.

2.4. Measurement parameters and odour classifiers

Twenty-four measurement parameters (features), shown in Table 2, were extracted from the response curves (Fig. 2). Multiclass linear discriminant analysis (LDA) was then used to test the significance ($P < 0.05$) of differences between odour classes across the selected features and to find the linear combinations of feature measurements which best separated the classes (Scott et al., 2007).

In LDA (McLachlan, 2004; Di Natale et al., 2006) the coefficients for these linear combinations of the p original (feature) measurements are calculated from the data, with m-1 sets of coefficients if there are m classes and $m < p$. Each set of coefficients defines a new variable and the new

Table 1
Details of odour sampling experiments.

Experiment	Sampling time (s)	Odour sample type	Number of sheep	Number of replicates
1	1200	Dry wool	4	34
	1200	Day 0 flystrike	2	13
	1200	Day 1 flystrike	2	19
	1200	Day 2 flystrike	2	19
	1200	Day 3 flystrike	2	11
	1200	Day 0 faeces	1	7
	1200	Day 1 faeces	1	8
	1200	Day 2 faeces	1	9
	1200	Day 3 faeces	1	9
	1200	Day 2 urine	1	10
2	1200	Dry wool	2	10
	1200	Day 0 flystrike	1	6
	1200	Day 1 flystrike	1	11
	1200	Day 2 flystrike	1	9
	1200	Day 3 flystrike	1	6
3	60	Dry wool	2	8
	60	Day 0 flystrike	1	9
	60	Day 1 flystrike	1	10
	15	Day 1 flystrike	1	1
	10	Day 2 flystrike	1	5
	6	Dry wool	1	7
	5	Day 2 flystrike	1	6
	2	Day 3 flystrike	1	10
4	60	Dry wool	1	8
	60	Day 0 flystrike	1	8
	60	Day 1 flystrike	1	9
	60	Day 2 flystrike	1	8
	60	Day 3 flystrike	1	8

Table 2
Features extracted from e-nose response curves for pattern recognition analysis.

	Feature calculation		Feature calculation
1.	$V_B + V_U$[a]	13.	$av(V_X{:}V_Y) - V_O$
2.	$V_A - V_O$	14.	$av(V_L{:}V_M) - V_O$
3.	T_R	15.	$av(V_K{:}V_M) - V_O$
4.	T_I	16.	$av(V_K{:}V_M)/(T_M - T_K)$
5.	$av(V_R{:}V_I) - V_O$	17.	$av(V_X{:}V_Z)/(T_Z - T_X)$
6.	$(V_I - V_R) - V_O$	18.	$(V_Z - V_Y) - V_O$
7.	$\min(V_R{:}V_I)$	19.	$(V_Y - V_X) - V_O$
8.	$\max(V_R{:}V_I)$	20.	$(V_M - V_L) - V_O$
9.	$(V_N - V_H)$	21.	$(V_L - V_K) - V_O$
10.	$T_I - T_R$	22.	$(V_M - V_K)/(T_M - T_K)$
11.	$(V_N - V_H)/(T_I - T_R)$	23.	$(V_Z - V_X)/(T_Z - T_X)$
12.	$av(V_R{:}V_I)/(T_I - T_R)$	24.	$av(V_X{:}V_Y) - V_O$

[a] V = potential (mV); T = time (s); av = average; min = minimum; max = maximum; References: B = baseline; U = signal start; A = maximum amplitude; O = offset; R = signal recognition; I = signal inflection; H = minimum; N = maximum; K = maximum T_R of single channel between first and last replicate; L = minimum T_I of single channel between first and last replicate; M = maximum T_I of single channel between first and last replicate; X = maximum T_R of MOS channels between first and last replicate; Y = minimum T_I of MOS channels between first and last replicate; Z = maximum T_I of MOS channels between first and last replicate.

variables can be used to display differences between groups graphically as well as to provide the basis for statistical tests of the differences and classification rules for new observations. The method assumes the within-class variance–covariance matrix is the same for all classes.

The first set of coefficients is chosen to maximise the ratio of its mean square between classes to its mean square within classes and, to ensure uniqueness, to satisfy a normalisation condition, for example that the corresponding new variable has unit sample variance. Here, if S_b and S are the estimated variance–covariance matrices between and within odour classes for the 24 feature measurements, c_1 is the vector of coefficients for the first linear combination of the measurements and c_1^T its transpose, then the elements of c_1 will maximise the ratio $c_1^T S_b c_1 / c_1^T S c_1$. The second set of coefficients is chosen in the same way, but subject to the additional constraint that the new variables from c_1 and c_2 are uncorrelated. The other sets are similarly constructed. Mathematically $c_1, c_2, \ldots$ are eigenvectors of $S^{-1}S_b$.

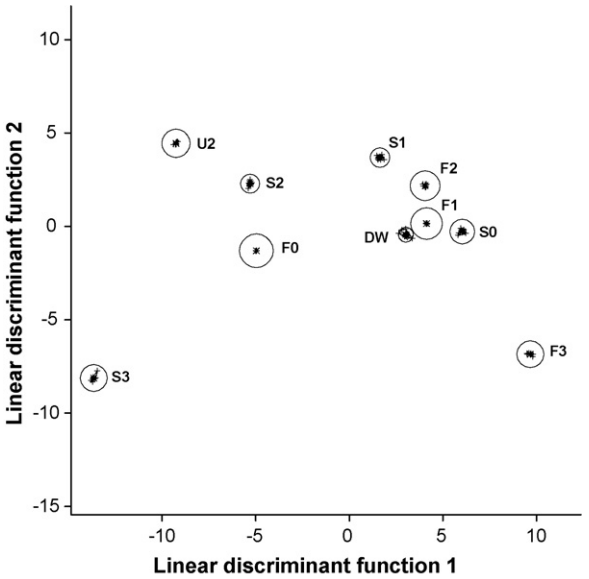

Fig. 3. Discriminant analysis of odour collection data from experiment 1 (DW = dry wool, S0 = 0–6 h flystrike odour, S1 = 24–30 h flystrike odour, S2 = 48–54 h flystrike odour, S3 = 72–78 h flystrike odour, U2 = 48–54 h urine odour, F0 = 0–6 h faeces odour, F1 = 24–30 h faeces odour, F2 = 48–54 h faeces odour, F3 = 72–78 h faeces odour). Circles surrounding clusters of points represent 95% confidence limits from the centre of the point distribution.

3. Results

An example of the raw output from the electronic nose is shown in Fig. 2. There are six MOS non-linear response curves and output lines from the humidity and temperature sensors. Peaks correspond to the exposure to flystrike odour and then declines to flushing with reference air for 20 min between exposures.

The results of the LDA for the four experiments are shown in Figs. 3–6. The circles surrounding the data

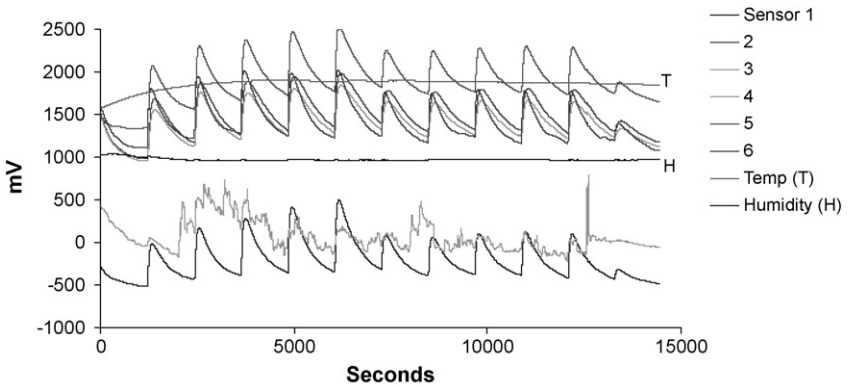

Fig. 2. Raw output from e-nose sensors. The peaks in lines 1–6 represent the responses of different MOS sensors to flystrike odour. The two relatively flat lines, 7 and 8, are from the temperature and humidity sensors.

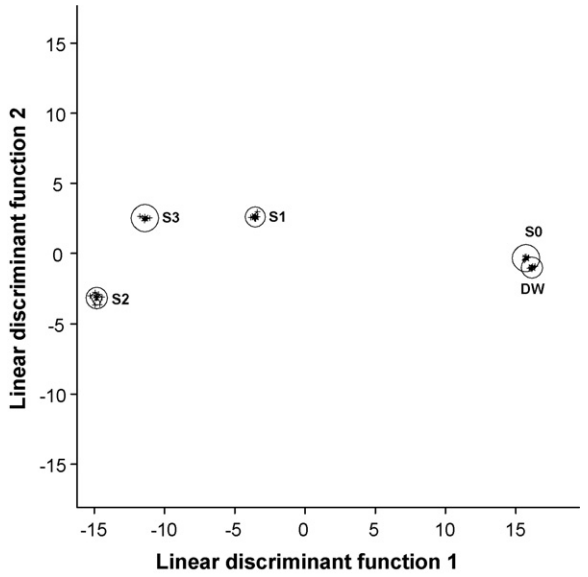

Fig. 4. Discriminant analysis of odour collection data from experiment 2 (DW = dry wool, S0 = 0–6 h flystrike odour, S1 = 24–30 h flystrike odour, S2 = 48–54 h flystrike odour, S3 = 72–78 h flystrike odour). Circles surrounding clusters of points represent 95% confidence limits.

clusters indicate the 95% confidence limits. Where circles around data clusters do not intersect, the odours are significantly discriminated ($P < 0.05$). Points most distant from each other are most confidently distinguished. Where there appears to be only a single point within the 95% confidence limit, as occurs for some odours in Figs. 3 and 4 this is because the points for different sensing periods clustered very tightly and overlaid each other when plotted.

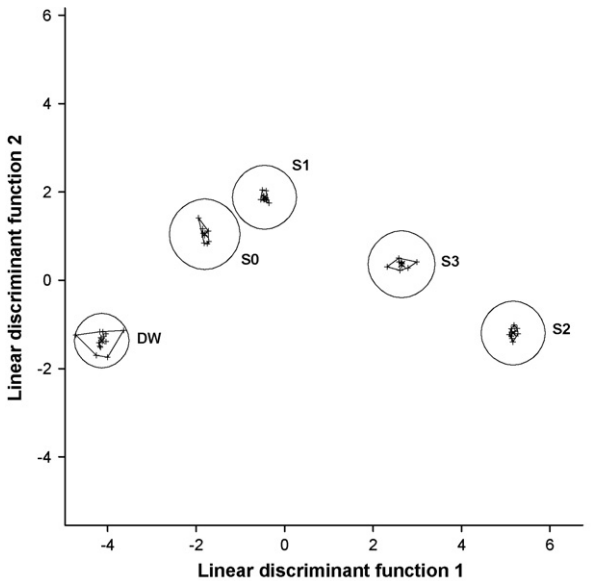

Fig. 5. Discriminant analysis of odour collection data from experiment 3 (DW = dry wool, S0 = 0–6 h flystrike odour, S1 = 24–30 h flystrike odour, S2 = 48–54 h flystrike odour, S3 = 72–78 h flystrike odour). Circles surrounding clusters of points represent 95% confidence limits.

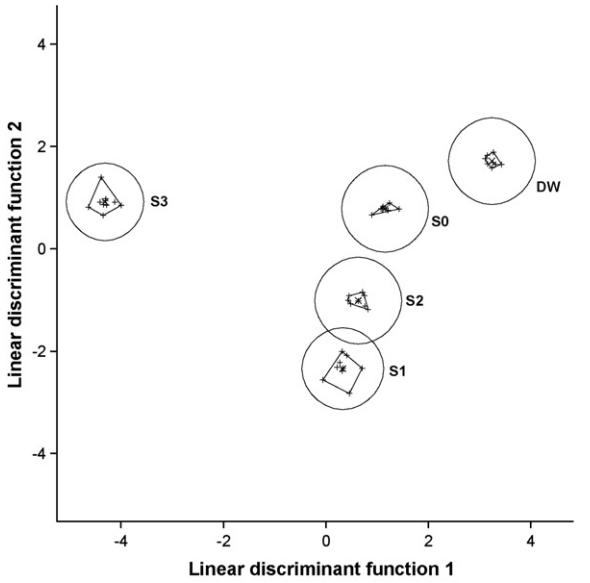

Fig. 6. Discriminant analysis of odour collection data from experiment 4 (DW = dry wool, S0 = 0–6 h flystrike odour, S1 = 24–30 h flystrike odour, S2 = 48–54 h flystrike odour, S3 = 72–78 h flystrike odour). Circles surrounding clusters of points represent 95% confidence limits.

The e-nose accurately discriminated dry wool from the strike odours of days 1–3 in all four experiments (Figs. 3–6) and was also able to accurately distinguish dry wool from the odour of strike on the day on which first instar larvae were implanted (measured up to 6 h after implantation) in all experiments except for experiment 2. In addition, in experiment 1, the electronic nose accurately distinguished the odour of urine stain and faecal stain from both flystrike and dry wool (Fig. 3).

4. Discussion

For utility as a flystrike detection device, the electronic nose must be able to reliably distinguish flystrike odours from sheep odour as well as from other odours that are likely to be encountered. In all four data sets, the electronic nose reliably discriminated the odour of flystrike from that of unstruck sheep. It is particularly notable that in all four data sets the electronic nose could identify strike within 24 h of application of the implant. This is earlier than clinical signs would usually be apparent, and probably much earlier than strike would normally be detected by paddock inspections. Wardhaugh and Dallwitz (1984) described a high prevalence of strikes that would not be detected by normal inspection, which they termed covert strikes. These strikes sometimes developed into full blown strikes but in some instances continued as covert strikes, undetected for some time, causing pain to the sheep and possibly providing a mechanism of maintenance for fly populations. Indications to date are that electronic nose technology may be able to identify the presence of these strikes enabling their early treatment.

In experiment 1, urine and faeces stain were also included in the study. These are conditions commonly encountered in practical sheep production systems, are

important predisposing conditions for flystrike and produce odours that could potentially be confused with strike. The electronic nose reliably distinguished strike odours from these two conditions.

Under practical conditions, the electronic nose may be required to detect the presence of strike with a very limited exposure to the odour emitted from a struck sheep. The most likely scenario is with the electronic nose positioned in a raceway through which sheep must pass, for example to obtain access to water. A hand held device to detect struck sheep in a mob has also been suggested. As this study was conducted to provide proof-of-concept for the use of electronic nose technology for flystrike detection, in the early experiments, flystrike odour was collected immediately above the strike and the period of 'sniffing' the strike odours was prolonged in order to give maximum opportunity for odour detection. However, in experiment 3 where the period of sniffing was only 2 s in one instance and in experiment 4, where air was collected from a chamber housing the sheep with the intake 0.7 m away from the sheep the electronic nose still gave accurate discrimination of strike.

Automated detection of flystrike has a number of significant potential benefits including cost and labour savings through reduced flock monitoring and reduced preventative treatments, animal welfare benefits through early strike detection and treatment, reduced chemical residues, lowered occupational health and safety risk through reduction in chemical treatments and reduced selection for resistance in fly populations through better targeted use of chemicals. This study provides proof-of-concept for the detection of flystrike using electronic nose technology.

More extensive experiments are now required to test the system under a wider set of circumstances. The collection of data under a range of conditions will be required for the development of optimal detection algorithms and there will be engineering challenges in the design of practical sheep delivery and odour collection systems to enable remote operation. However, with the rapid advances currently being made in electronic nose technology, solar power and communication systems, the vision of remote strike detection technology that can notify managers of the presence of struck sheep in the mob, or even potentially interface with E-sheep technology (Rowe, 2006) to draft off struck sheep, seems realistic.

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