# Behaviour as a means of differentiating healthy from sub-clinically ill cattle

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#### Abstract

**Aims** While behavioural change associated with clinical disease in cows has been widely studied, the impact of subclinical disease on behaviour is less known. The ability to detect subclinical disease via behavioural changes using targeted monitoring could help to improve cow welfare and decrease economic loss.

**Methods** This study investigated the possibility of assigning a cow as a healthy control or having subclinical mastitis based purely upon behaviour, by comparing 24 hours of an 'unclassified' cow's behavioural data to reference data and checking the prediction.

**Results** Predictions were accurate when using the 24-hour data set, but classification using specific time periods or behaviours may also be possible, with view of future automation of the recording, identification and classification of behaviours, which is currently all done manually.

**Conclusions** Targeted monitoring would be less time-consuming than 24-hour monitoring and better suited for routine cow-health monitoring. To validate these initial findings, further investigation with more focal cows and a larger reference data set is required.

#### Introduction

Clinically diseased individuals often display sickness behaviour as part of a systemic inflammatory response to infection.<sup>1,2</sup> This can include reductions in physical activity, social behaviour and feeding/ drinking.<sup>3</sup> Although inflammatory markers can also be up-regulated in sub-clinical disease,<sup>4,5</sup> the impact of sub-clinical inflammation upon livestock behaviour and welfare remains largely unstudied.

This study formed part of a project aiming to detect behavioural differences between cows with and without subclinical mastitis (SCM). Currently, mastitis detection occurs during milking, as farmers pick up clinical signs and overt sickness behaviour. Since direct behavioural observations of focal cows are extremely time-consuming, we investigated the potential for targeting specific behaviours and/or discrete time periods (1 hour blocks versus 24 hours) by utilising reference baseline data previously collected from a commercial dairy herd to assign health status to a visually healthy cow.

#### Methods

A reference data set of behaviour had been previously compiled from 24-hour video footage (in 1-hour blocks, long enough to display infrequent behaviours) from pregnant dairy cows without clinical symptoms, using a comprehensive ethogram (**Table 1**). The cows were assigned to two groups (n=12 control and n = 12 SCM; paired for parity, age, pregnancy state and milk yield). Cows in the

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SCM group had a somatic cell count (SCC) of >200,000 cells/ml at the time of data collection.

For this sub-study, continuous direct observations were also recorded over 24 hours for an 'unclassified' non-pregnant cow of unknown health status (the investigator was blind to the SCC count). Starting at 00:00 hours on the day of interest, the number of behavioural transitions was calculated for each time period and focal behaviours were recorded. Behavioural data was compiled into two sets: (1) hourly totals; and (2) 24-hour totals for all behaviours (and transitions). No data was recorded at 06:00 hours, 14:00 hours or 22:00 hours, when the cow was in the parlour. When the cow was not visible for 3600 seconds/hour, the behavioural data was adjusted by protracting the duration of the behaviours proportionally over 3600 seconds (=3600/ seconds visible×behaviour duration), providing there were over 1800 seconds of footage in which the cow was visible, otherwise the hour was discarded entirely. The '24 hour total' data set was also adjusted to reflect the duration (seconds) or number (n) of each behaviour 'per hour of total time visible'.

Paired *t* tests were performed, using SPSS, on each behavioural measure within the reference data set. For measures that showed a significant difference between 'groups' (p<0.05), 95% confidence intervals (Cls) were calculated to produce estimates of diagnostic range **(Table 2)**. These diagnostic parameters were then used to assign the 'unclassified' cow to a group (control or SCM).

#### Results

Of the behaviours analysed, 23/36 were statistically significant in categorising control vs SCM data. Using these 23 behaviours, the '24 hour total' reference data predicted the unclassified cow would fall within the control group: 16/23 behaviours fell within control parameters, 5/23 within SCM, and 2/23 were unclassified (**Table 2**). In contrast, categorisation of the unclassified cow considering the behavioural data for individual hours was not possible: 20/41 fell within control parameters, 17/41 within SCM, and 4/41 were unclassified (**Table 2**).

SCC data (SCC = 63,000 cells/ml) confirmed the unclassified cow was a control. Although the continuous observation of 24 hours of behavioural footage enabled us to correctly assign the cow as SCM-free, it was time consuming and labour intensive.

Overall, the division of data into hourly blocks for analysis did not improve correct group assignment. However, there is an indication that 00:00-01:00 hours may be a targetable time regarding social behaviour (or lack, thereof), since 'explore social', 'social all give' and 'social all receive' correctly classified the cow during this period (**Table 2**). Feeding behaviour and activity levels (measured by behavioural 'transitions') may also prove useful as key behaviours to log at night/ early morning: 'feed' correctly classified the cow within the intervals of 23:00 to 00:00 hours and 02:00 to 03:00 hours, whilst 'transitions' led to correct classification between 02:00 to 03:00 hours and 05:00 to 06:00 hours (**Table 2**).

## Table 1. Data from ethogram recording of cow behaviour, including direct behavioural observations, broader descriptive categories and units of measure.

Behaviour	Unit of measure	Definition		
Observed behaviour				
Allogroom give	n/s	Licking a conspecific		
Allogroom receive	n/s	Receiving licks from a conspecific		
Body push give	n/s	Sideways shunt (e.g. when entering crowded area of feed passage barrier) delivered by flank of focal cow		
Body push receive	n/s	Receipt of body push by conspecific		
Brush	s	Use of the mechanical brush		
Challenge give	n/s	A threatening non-contact gesture towards a conspecific (e.g. a determined approach with a head-down posture)		
Challenge receive	n/s	Receipt of a challenge from a conspecific		
Chin press give	n/s	Exerting chin pressure on the lateral posterior of a conspecific		
Chin press receive	n/s	Receipt of a chin press by a conspecific		
Drink	s	Ingestion of water		
Feed	s	Actively ingest/chew food (non-rumination)		
Eliminate	s	Defecate or urinate		
Explore cow	n/s	Sniffing a conspecific without physical contact		
Explore food	S	Sniffing/nosing food without obvious ingestion		
Explore pen	s	Sniffing or licking any part of the barn structure		
Explore sand	s	Sniffing a sand bed		
Head butt give	n/s	Violent striking of a conspecific using a lowered head		
Head butt receive	n/s	Receipt of a head butt from a conspecific		
Head push give	n/s	Using the head to gently push/nudge a conspecific		
Head push receive	n/s	Receipt of a head push from a conspecific		
Head swipe give	n/s	Sideways swipe of head (usually directed at a conspecific's head)		
Head swipe receive	n/s	Receipt of sideways head swipe from conspecific		
Lick self	s	Licking own body		
Lie_head down	s	Head resting on floor/cubicle bar whilst lying (no rumination)		
Lie_head on flank	s	Head held against the flank (pointing towards rump) whilst lying		
Lie_head up	s	Head held upright whilst lying (with/without rumination)		
Lying	s	Shift in vertical orientation from standing to lying		
Mutual head butt	n/s	Mutual head butting between the focal cow and a conspecific		
Mutual head rub	n/s	Mutual head rubbing between the focal cow and a conspecific		
Mutual sniff	n/s	Mutual sniffing between the focal cow and conspecific		
Rising	s	Shift in vertical orientation from lying to standing		
Rub self	s	Rubbing body upon pen furniture		
Run	s	Running		
Scratch self	s	Scratch body with leg		
Stand	s	Standing		
Walk	s	Walking		
Calculated behaviour	l			
Comfort	s	Brush + lick self + rub self + scratch self		
Explore non-social	s	Explore food + explore pen + explore sand		
Explore social	n/s	Explore cow + mutual sniff		
Lie all	s	Lie with head down + lie with head up + lie with head on flank		
All (non-affiliative) social give	n/s	Body push give + challenge give + chin press give + head butt give + head push give + head butt		

s, seconds

Table 2: The classification of cow of 'unknown' health status as being a control (Ctrl) or as having SCM using behavioural data and a reference data set taken from the same herd.

	Unit		Refere	Unclassified cow			
Behaviour		Con	trol	sc	м		
		Diagnostic parameter	95% Cl	Diagnostic parameter	95% CI	Value	Classification
4 hour total							
Body push give	n	>0.62	0.43, 0.64	<0.43	0.29, 0.62	2.13	Ctrl
Brush	S	>24.2	7.2, 33.5	<6.78	6.8, 24.2	34.8	Ctrl
Challenge give	n	>0.16	0.08, 0.30	<0.08	0.03, 0.16	1.3	Ctrl
Challenge receive	n	>0.31	0.22, 0.51	<0.22	0.12, 0.31	1	Ctrl
Chin rest receive	n	ND	0.01, 0.05	>0.05	0, 0.22	0.1	SCM
Comfort	S	<28.9	28.9, 59.3	>51	29.4, 51	56.5	SCM
Explore cow	n	>1.32	0.84, 1.66	<0.84	0.70, 1.32	4.2	Ctrl
Explore food	S	>17	12.4, 23.8	<12.4	11.1, 17	32.9	Ctrl
Explore sand	S	>29.9	22.2, 35.9	<22.2	16.9, 29.9	28.8	Unclassified
Explore non- social	S	>51.2	42, 65.2	<42	38.1, 51.2	74.6	Ctrl
Explore social	n	>1.65	1.04, 1.94	<1.04	0.86, 1.65	4.2	Ctrl
Explore social	S	>15.6	7.12, 17.7	<7.12	6.77, 15.6	60.2	Ctrl
Feed	S	<843	793, 935	>935	843, 1070	471.4	Ctrl
Head butt give	n	>0.99	0.45, 1.06	<0.45	0.35, 0.99	2.1	Ctrl
Head butt receive	n	>0.75	0.49, 0.94	<0.49	0.46, 0.75	0.3	SCM
Head push give	n	>0.15	0.07, 0.16	<0.07	0.03, 0.15	1.9	Ctrl
Head push receive	n	>0.13	0.08, 0.27	<0.08	0.02, 0.13	0.5	Ctrl
Head swipe give	n	<1.68	1.08, 1.86	>1.86	1.68, 2.98	2.7	SCM
Head swipe receive	n	<1.43	1.07, 2.73	>2.73	1.43, 2.97	0.5	Ctrl
Lie_head on flank	S	<145	126, 209	>209	145, 232	155.3	Unclassified
Rub self	S	>8.61	4.82, 8.88	<4.82	3.55, 8.61	15.8	Ctrl
All social give	п	<3.11	3.10, 4.45	>4.45	3.11, 5.25	11.6	SCM
Transitions	n	>53.6	49.7, 57.7	<49.7	43.5, 53.6	64.7	Ctrl
dividual hour							
Brush—23:00 hours	S	<0.49	0, 0.91	>0.91	0.49, 30.7	0	Ctrl
Drink—12:00 hours	S	>34.3	3.9, 67.7	<3.9	14.8, 34.5	0	SCM
Drink—15:00 hours	S	>117	78.1, 157	<78.1	25.5, 117	151.2	Ctrl
Drink—18:00 hours	S	>45.9	17.7, 87.1	<17.7	0, 45.9	0	SCM
Explore non- social—05:00 hours	S	>17.4	0, 198	ND	1.21, 17.4	204	Ctrl
Explore non- social—16:00 hours	S	>85.8	59, 134	<59	32, 85.8	106	Ctrl
Explore non- social—21:00 hours	S	<25.8	12, 42.2	>42.2	25.8, 75.8	7	Ctrl
Explore non- social—23:00 nours	S	>83.4	44.1, 132	<44.1	23.6, 83.4	82	Unclassified
Explore social—00:00 nours	S	<3.4	0.12, 7.38	>7.38	3.4, 18.6	0	Ctrl
Explore social—02:00 nours	S	>6.9	0, 44.5	ND	0, 6.9	57	Ctrl
Explore social—05:00 hours	S	>0	0, 37	ND	0	158	Ctrl

Explore social—16:00 hours	S	<12.2	1.04, 13.5	>13.5	12.2, 89.8	195.8	SCM
Feed—02:00 hours	5	>425	0, 491	ND	0, 425	554	Ctrl
Feed—18:00 hours	S	>446	86.8, 1240	<86.8	0, 446	0	SCM
Feed—23:00 hours	S	<1300	332, 1300	>1390	1390, 2780	373	Ctrl
Lie_all—05:00 hours	S	<3200	2150, 3240	>3240	3299, 3480	3495	SCM
Lie_head on flank—00:00 hours	5	>220	66.8, 533	<66.8	0, 220	11	SCM
Lie_head on flank—11:00 hours	5	<36.1	16.1, 226	>226	36.1, 376	124	Unclassified
Lie_head on flank—13:00 hours	S	<39.7	0, 142	>142	39.7, 438	249	SCM
Lie_head on flank—23:00 hours	S	>0	0, 201	ND	0	169	Ctrl
Social all receive—00:00 hours	S	<7.52	2.4, 19.7	>19.7	7.5, 94.3	0	Ctrl
Social all receive—02:00 hours	n	>0.93	0.62, 5.88	<0.62	0, 0.93	0	SCM
Social all receive—02:00 hours	S	>1.85	1.4, 17.1	<1.43	0, 1.9	0	SCM
Social all receive—05:00 hours	п	>0.24	0.20, 1.54	<0.20	0, 0.24	0	SCM
Social all receive—08:00 hours	n	<5.09	2.6, 8.1	>8.14	5.09, 12.6	0	Ctrl
Social all receive—12:00 hours	n	>5.38	1.8, 12	<1.83	1.12, 5.38	5	Unclassified
Social all receive—15:00 hours	S	>37.4	30.1, 62.4	<30.1	17.2, 37.4	277.7	Ctrl
Social all receive—18:00 hours	n	>2.28	0.4, 12.1	<0.4	0.22, 2.28	0	SCM
Social all receive—23:00 hours	n	<5.64	2.8, 8.3	>8.29	5.64, 15.5	8	Unclassified
Social all give—00:00 hours	S	<1.62	1.6, 15.6	>15.6	1.6, 92.2	0	Ctrl
Social all give—04:00 hours	n	>0.391	0, 2.7	ND	0, 0.39	26	Ctrl
Social all give—04:00 hours	S	ND	0	>0	0, 2.5	512	SCM
Social all give—07:00 hours	n	<5.82	2.5, 7.7	>7.74	5.82, 14.5	45	SCM
Social all give—11:00 hours	n	<1.62	0.8, 4.9	>4.9	1.62, 11.7	19	SCM
Social all give—16:00 hours	п	<3.64	3.1, 6.6	>6.58	3.64, 12.4	53	SCM
Social all give—23:00 hours	n	<4.5	1.7, 6.4	>6.43	4.5, 11.3	17	SCM
Allogroom give—16:00 hours	S	<6.7	0, 88.8	>88.8	6.7, 128	5.4	Ctrl

Allogroom receive—15:00 hours	S	<1.3	0	>1.3	1.3, 101	17.6	SCM
Allogroom receive—16:00 hours	S	>23	8.7, 50.9	<8.73	0, 23	34.6	Ctrl
Transitions —02:00 hours	n	>40.7	8.5, 97.9	<7.67	7.7, 40.7	42	Ctrl
Transitions —05:00 hours	n	>18.4	18.4, 40.4	<16.2	6, 16.2	19	Ctrl

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ND, no data; s, seconds

#### Discussion

The possibility of allocating an unclassified cow to a control group based upon 24 hours of continuous observations has been demonstrated. This study provides initial evidence that night/early morning may be interesting times to monitor specific behaviours (social behaviour, feeding and activity) for diagnosing SCM in dairy cattle. Some concerns addressed by this project were ensuring inter-observer reliability (best when using qualitative behaviour assessment)<sup>6</sup> and utilising a comprehensive and unambiguous ethogram.7 Since the reference data set was compiled using a relatively small sample (n=24), including data from more cows will probably increase predictive capacity. Parlour behaviour was not included as the view was to identify freely displayed behaviours suitable for automated video/collar analysis. Hierarchy (possibly influenced by health) was not considered in relation to behaviour variation. Hopefully, in the future, behavioural analysis, combined with non-invasive monitoring of saliva acute-phase proteins (indicative of low-level systemic inflammation<sup>5</sup>), will enable routine herd monitoring for subclinical disease, benefitting farm profitability and cow welfare.

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