APPENDIX

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Learning from critical care management of sheep receiving extra-corporeal membrane oxygenation for smoke-induced acute lung injury as a tool for processing large clinical datasets

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Abstract

Background: Many successful therapies developed for human medicine involve animal experimentation. With competition for public research funding and career advancement opportunities, animal studies focused on the translational potential may not sufficiently document unexpected outcomes. Such studies often have hastily developed methods with *ad hoc* modifications including the use of additional animals, leading to considerable amounts of idle, unprocessed data that could be used to advance veterinary science, or to refine the base animal model. Sheep, for example, are poorly understood models of intensive care and therefore, any experimental data arising from them should be interpreted with care. The hypothesis was that there is little information describing the development of methods of physiological data processing in multifaceted sheep models of intensive care and the author aimed to develop a suitable data processing method and to analyse the data, once processed.

Methods: Data from 19 adult mechanically ventilated ewes undergoing intensive care in a previous study evaluating a form of extracorporeal life support (treatment) for acute lung injury were used to develop a comprehensive method for processing manual and electronically gathered clinical observations. Eight sheep were injured by acute smoke inhalation prior to treatment (injured/treated), while another eight were not injured but treated (uninjured/treated). Two sheep were injured but not treated (injured/untreated), while one received room air instead of smoke as the injury, and was not treated (placebo/untreated). The data were then analysed for 11 physiological categories and compared between the two treated groups.

Results: The analysis revealed that compared with the baseline, treatment contributed to and exacerbated the deterioration of pulmonary pathology by reducing lung compliance and PaO_2/FiO_2 ratio. The oxygen extraction index changes mirrored those of the PaO_2/FiO_2 ratio. Decreasing coronary perfusion pressure predicted the severity of cardiopulmonary injury.

Conclusions: These novel observations could help in understanding similar pathology such as that which occurs in smoke inhalation animal victims of house and bush fires. A similar data processing method could be used when evaluating the effectiveness of other clinical interventions such as potentially reversible aspiration pneumonia secondary to tick paralysis in veterinary patients.

Keywords: Sheep, critical care, smoke-induced acute smoke inhalation injury, extra-corporeal life support, lung compliance, PaO_2/FiO_2 ratio

Background

During multifaceted experiments involving intensive care in large animal models in translational research, information regarding animal monitoring is often collected with varying accuracy, scope, and end-user applications. Data collection can be manual, electronic, or both [1-3]. Manually input data can include subjectively scored end-points such as the plane of anaesthesia, and objective data such as heart rate or breaths per minute. Depending on the goals of the study, some information may be used to validate or test novel therapies, or to understand and refine existing treatments. In some cases, experimental information may be gathered for scientific curiosity or for "classified" use, and outcomes may never be publicly available, especially if the results are negative.

The source of data for this study was from a sheep model [2-4] being treated for smoke-induced acute lung injury using veno-venous extracorporeal membrane oxygenation [2], a form of extracorporeal life support (ECLS) developed to complement the treatment of acute lung injury in humans [5-7]. During this type of ECLS, venous blood is carried from the patient to a gas exchange device where it becomes enriched with oxygen, has carbon dioxide removed, and is returned to the patient's circulation in the right heart. This method can be used for treatment, as respiratory support during lung transplantation, and in critically ill patients with potentially reversible respiratory failure [7]. The multiple advanced cardiovascular [3], respiratory, patient point-of-care procedures, and instrumentation associated with ECLS, even in animal experimentation, is highly data- and equipment-intensive. This platform is useful for developing research and methodological skills for *in vivo* animal instrumentation and the processing of large, real-time clinical data sets from multifaceted animal studies that can be applied to similar intensive care scenarios. An opportunity to develop these skills arose within the source study, which was an ongoing publicly funded animal experimentation study (Queensland University of Technology Animal Ethics Approval No. 110000053). While the objectives of the primary study had a separate focus, there were considerable amounts of redundant raw data with potential use in veterinary science and other disciplines, once processed. The author hypothesised that there was little information describing the development of methods of physiological data processing in multifaceted large animal models of intensive care.

The overall goal was to provide useful information relevant to the sheep model, itself, and to those interested in large animal experimentation and veterinary medicine, generally. The specific objectives were: 1) use the raw data from the sheep model study to create a data management system for tabulating large data sets from human studies using animal models and, 2) analyse that data to provide biological information that is not currently available for sheep receiving ECLS following smoke-induced acute lung injury.

Methods

The study was carried out at the purpose-built Medical Engineering Facility of Queensland University of Technology (QUT-MERF) at the Prince Charles Hospital Campus of The University of Queensland [1]. In the original study, sheep inhaled standardised cotton smoke generated by a device that combusts material in an oxygen-deficient environment as previously described [8]. Briefly, 8 g of cotton towelling was combusted in a chamber with transparent walls and 400 ml tidal volume. One tidal volume breath (approximately 10–12 ml/kg) of the smoke was delivered to the sheep via plastic tubing connected to a 1-m-long tracheostomy tube. A fixed number (12) of breaths were given with each load of cotton over a period of approximately one minute. Serial arterial blood gas samples were taken to assess the effect of smoke inhalation, starting at a predetermined time point after the smoke breath cycles.

Physiological data management system

Raw data were obtained from a previous translational study of critical care monitoring of sheep undergoing treatment for smoke-induced acute lung injury involving several separate projects. Data were collected prior to 23 August 2013 and were obtained from two of the scientists who developed the base model [4] as part of a Research Higher Degree project of the author [9, 10]. All data files were in Microsoft® Excel 97–2003 (Microsoft Corporation, Redmond, WA, USA) format and were grouped per sheep and date of the experiment. Data consisted of separate files of real-time physiological data recorded on the hard drives of the monitoring devices (electronically acquired data), and parameters manually recorded by those monitoring the sheep under anaesthesia (manually acquired data), which included data from the electronic monitoring equipment, as back-up if the electronic monitor malfunctioned.

The source study involved 64 sheep, comprising eight experimental groups of eight sheep based on the study's multiple objectives, subsequent modifications, and later addition of experimental controls. The experimental groups were classified based on: the duration of treatment (2 and 24 hours; E2H and E24H); treatment after smoke inhalation (injury) for 2 and 24 hours (SE2H and SE24H); and treatment after smoke inhalation and transfusion with fresh or stored (aged) blood (SEF24H or SEA24H), respectively. Two additional groups included one group receiving smoke inhalation injury but no treatment (SC24H), and another group that inhaled room air only as the injury (placebo) and no treatment (C24H). Data from sheep involved in the treatment and transfusion studies were not included in the analysis in this study because these data were beyond the scope of this study. Nineteen sheep were included in the present study; data were analysed for 16 sheep with robust data (E24H and SE24H), and included, but not analysed, for three from groups

SC24H and C24H (early observational data). A systematic approach was developed for processing the data.

Manually acquired physiological data workflow

A clone of the master manual data entry spreadsheet was created by removing the formatting and formulas. Several members of the sheep ECMO research team inspected data repeatedly for errors to ensure that all columns, rows, time points, and data points had been copied correctly, including number formats (Figure 1). Redundant columns were removed and data were aligned to experimental time points (Figure 2). While maintaining the same experimental time point header, data from the table in Figure 2 were split and grouped into the following categories: ventilator settings, blood pressure and haemodynamics, fluids and urine output, arterial blood gas values, activated clotting time, anaesthetics, anticoagulants, and ECLS circuit observations.

Electronically acquired physiological data workflow

Raw electronically acquired physiologic monitoring data were inspected for completeness. The data comprised 36 time points: ECLS pump time (min); time of day (h); electrocardiograph (heart rate); arterial blood pressure (mean, systolic, diastolic, heart rate); central venous pressure (mean); pulmonary artery pressure (mean, systolic, diastolic); oxygenator pressure (pre- and post-); capnography (end-tidal carbon dioxide ($etCO_2$), respiratory rate); pulse oximetry (SpO_2, heart rate); ECLS pump (flow rate, speed); ventilator (mode, frequency, oxygen, pressure control, inspiratory volume, expiratory volume, expiratory minute volume, pressure maximum, mean pressure, positive end-expiratory pressure, plateau pressure, inspiratory resistance, expiratory pressure, pulmonary compliance, inspiratory flow); mixed venous oxygen saturation (SvO_2); and continuous cardiac output (CCO) (Figure 3). The yellow line in Figure 3 indicates the baseline time point and the grey line represents the smoke inhalation time point. It is important to note that there may or may not have been any data at any given point in time.

The electronically acquired physiological monitoring data were inspected for errors and cleaned to provide data for downstream analysis (Figure 4).

Pre-data analysis checks

Data were then subjected to further integrity checks. An important step was to make a plot of data versus time together with descriptive statistics for all data points in the grouped data. At the time of data processing, the “Descriptive Statistics” tool of Microsoft® Excel 2010 (Microsoft Corporation) did not complete analysis with missing values. Therefore, the data to be analysed were selected and then the “GoTo” tool (F5) was used, and “Special”, “Blanks”, and thereafter, “OK” were selected to

identify blanks. The blanks were deleted by positioning the cursor in the blank cell and using the space bar to clear the cell (the “Delete” or “Backspace” keys did not remove the blanks).
After artefact removal and integrity checks, data for individual sheep were placed into six categories: activated clotting time; anaesthetics + inotropes and anticoagulants; arterial blood gas values; blood pressure + ventilation and haemodynamic data; calculated respiratory + haemodynamic variables; and fluids and urine production. Using specially written macros, data were extracted from each experiment and grouped by parameter corresponding to experimental time points. All sheep treatment data were filed by parameter.
Data from the 19 sheep from groups E24H, SE24H, C24H and SC24H (Figure 5) were processed further. Data integrity checks were again performed and repeated by several sheep ECMO research team members, and data were compiled as shown in Figure 6 (note the obliterated cells after removal of data artefacts). The treatment timeline (a) comprised 22 time points for all experiments where sheep received smoke inhalation acute lung injury (SE24H) (b). A trend plot (c) and descriptive statistics panel (d) were useful in data quality control processes for suitability for downstream data analysis and end-user applications.

Statistical methods

To meet the second objective, data from the groups, uninjured/treated and injured/treated groups were analysed. The means, medians and standard deviations of the weights of the sheep, where applicable, were tabulated. The physiological parameters of the groups were charted and compared against each other using one-way analysis of variance (ANOVA), where appropriate. Parameters between groups were compared using a paired two-tailed t-test. All p-values were two-sided and $p < 0.05$ was considered statistically significant. All statistical calculations were performed using GraphPad PRISM 6 software (GraphPad Software, La Jolla, CA, USA).

Results

The biodata of the sheep that were used in the current analysis are presented in Table 1. The weights of the uninjured/treated sheep, unlike the injured/treated group, did not pass the D’Agostino–Pearson omnibus normality test; however, there was no significant difference in the weights of the sheep between groups.

Ventilation

All animals were intubated and received mechanical ventilation as previously described [1]. Briefly, the initial ventilator tidal volume was set to approximately 10 mL/kg with a respiratory rate of 15 breaths/min, positive end expiratory pressure (PEEP) of 5 cm H_2O, and an initial F_iO_2 (fraction of

inspired oxygen) of 1.0. These settings were then titrated based on arterial blood gas results. A low tidal volume, high PEEP strategy was used to minimise ventilator-induced lung injury.
Pulmonary compliance decreased in all of the sheep during the course of the experiments, with the injured/treated (SE24H) animals having the most severe and drastic decrease followed by the uninjured/treated (E24H), injured/untreated (SC24) and placebo/untreated (C24) sheep in that order (Figure 7). There was a significant difference ($p = 0.0013$) in pulmonary compliance between uninjured/treated and injured/treated groups. The injured/treated sheep had consistently lower SpO_2 compared with the other groups, but there was no significant difference in SpO_2 readings between the groups. There was an initial increase in $etCO_2$ followed by a rapid decrease that lessened 15 minutes after the start of treatment. The $etCO_2$ of the injured sheep continued to trend downward and plateaued in the uninjured groups. There was a significant difference ($p = 0.0147$) in $etCO_2$ between the uninjured/treated and injured/treated groups.

Blood gases (arterial samples)

Blood pH varied between the groups. The placebo/untreated sheep had the highest pH while the injured/treated group had the lowest. There was a significant difference in pH between the uninjured/treated and injured/treated groups ($p = 0.0343$).
The pCO_2 in all but the uninjured/treated sheep increased initially before plummeting sharply, forming a shallow trough corresponding to 1 hour after the start of treatment, followed by a slight increase before stabilising in all sheep.
There was a gradual decrease in pO_2 in the treated groups of sheep from baseline before decreasing dramatically at the start of treatment with the injured sheep having the most profound decrease (Figure 8). However, there was no significant difference in pO_2 between the uninjured/treated and injured/treated groups.
Haemoglobin [Hb] concentration decreased slightly from baseline before gradually increasing in the injured sheep and remained relatively constant over time in the uninjured sheep. There was a significant difference in [Hb] between the uninjured/treated and injured/treated ($p = 0.0131$) groups.
The fraction of oxyhaemoglobin (FO_2Hb) decreased sharply with the lowest reading at 5 minutes post-injury before returning to near baseline levels within an hour of starting treatment. The injured/treated sheep had a considerably deeper trough in FO_2Hb level and there was a significant difference ($p = 0.046$) between troughs. There was no change in FO_2Hb for the uninjured sheep.
The fraction of carboxyhaemoglobin (FCOHb) increased sharply from baseline, peaking at approximately 5 minutes post-injury and decreased sharply thereafter to the start of treatment before gradually returning to near baseline levels at approximately 6 hours post-start of treatment in the injured sheep. The injured/treated sheep had a higher peak FCOHb than the injured/untreated sheep,

although the difference was not significant. There was no change in FCOHb for the uninjured sheep.

The fraction of methaemoglobin (MetHb) increased gradually from baseline, peaking at approximately 5 minutes post-injury and then gradually decreased to the start of treatment. This was followed by a gradual return to near baseline levels at approximately 6 hours post-start of treatment in the injured/treated sheep. There was no change in MetHb for the uninjured sheep. There was an initial subtle decrease in calculated haematocrit (Hct) before a steady increase in the injured sheep and relatively flat slopes for the uninjured sheep.

Electrolytes

The blood sodium concentration [Na^+] was relatively stable and there were no significant differences between groups.

There was an initial decrease in the blood calcium [Ca^{2+}] level, with the lowest point at approximately 1 hour past the start of treatment before levelling out thereafter in all groups. There was a significant difference in [Ca^{2+}] between the uninjured/treated and the injured/treated groups ($p = 0.0001$). The placebo/untreated and injured/treated groups maintained the highest and lowest levels of [Ca^{2+}], respectively, throughout the experiments.

Blood chloride [Cl^-] levels remained stable compared with baseline levels during the initial stages and then increased gradually thereafter.

The blood potassium concentration [K^+] initially decreased compared with baseline levels, reaching a minimum concentration 1 hour after starting treatment and then gradually increased with a peak at approximately 12 hours post-treatment start in all experimental groups. Although the injured/untreated and injured/treated sheep had higher [K^+] than the uninjured sheep, the differences were not significant.

Overall, the anion gap decreased gradually, reaching a relatively gentle slope at approximately 6 hours from the start of treatment and did not change significantly, thereafter. There was a gradual decrease in anion gap from baseline during the course of the experiments and there was no significant difference in anion gap between the uninjured/treated and injured/treated groups.

Metabolites

Although there was an increase in blood glucose level [Glu] for the injured/treated sheep after 6 hours of treatment, the change was not significant.

There was an initial decrease in lactate levels [Lac] 6 hours after the start of treatment, followed by a gradual increase for the injured sheep, especially for the injured/treated group. There was no significant difference in [Lac] between the treated groups.

Acid-base balance

There was an increase in the blood base levels [Base (ecf)] that peaked 1 hour post-treatment, followed by a gradual decrease in the untreated group. [Base (ecf)] in the treated groups remained at baseline levels to 1 hour post-start of treatment, before decreasing markedly in the injured/treated sheep. There was a significant difference ($p = 0.0257$) in [Base (ecf)] between the uninjured/treated and injured/treated groups.

Blood bicarbonate concentrations [HCO_3^-] increased initially in the untreated groups before decreasing gradually; however, levels remained higher compared with the treated sheep.

Haemodynamics

There was a gradual decrease in heart rate (HR) during the course of the experiments, with the placebo/untreated groups maintaining a higher HR compared with the injured/untreated, injured/treated, and uninjured/treated groups early in the experiments. There was no significant difference in HR between the uninjured/treated and injured/treated groups.

The mean arterial blood pressure (MAP) decreased early in the experiments before subsequently increasing gradually, peaking at approximately the treatment start time point, before gradually decreasing again in all but the placebo/untreated sheep. The injured/treated groups had a consistently lower MAP compared with the other groups and there was a significant difference in MAP ($p = 0.0058$) between the uninjured/treated and injured/treated groups.

The mean pulmonary artery pressure (MPAP) increased gradually, with the injured/treated group having a consistently higher MPAP. There was no significant difference in MPAP between the uninjured/treated and injured/treated groups.

There was an initial, subtle increase in central venous pressure (CVP) that peaked at approximately 1 hour post-injury followed by a decrease that stabilised at approximately 1 hour post-start of treatment. CVP levels in the injured/treated and placebo/untreated sheep were consistently higher and lower, respectively, during the course of the experiments. Mixed venous oxygen saturation (SvO_2) had a lower baseline before eventually rising to a relatively stable and higher level for the treated sheep, and a slightly lower level for the untreated sheep. The injured/untreated sheep maintained a consistently lower SvO_2 compared with the other groups.

Except for the placebo/untreated group, there was a decrease in continuous cardiac output (CCO) from baseline to approximately 1 hour post-start of treatment. There was a significant difference ($p = 0.0009$) in CCO between the uninjured/treated and injured/treated groups with CCO in the treated groups increasing sharply before plateauing, especially in the uninjured/treated group. There was also a subsequent gradual decrease in CCO in the injured/treated group.

Stroke volume (SV) began to increase 1 hour from the start of treatment for all groups, except for the injured/untreated group where levels remained relatively constant. SV in the injured/treated group began to decrease after 6 hours of treatment, while SV in the uninjured/treated and placebo/untreated sheep increased steadily before decreasing or levelling out after 12 hours or more of treatment. There were no significant differences in SV between.

Stroke volume index (SVI) began to increase 1 hour after of the start of treatment for all groups, except for the injured/untreated group, for which SVI remained relatively constant. SVI in the injured/treated group began to decrease after 6 hours of treatment while SVI in the uninjured/treated and placebo/untreated groups increased before subsequently decreasing or levelling out after 12 hours or more of treatment. There were no significant differences in SVI between groups.

While the cardiac index (CI) of the uninjured/treated and placebo/untreated groups remained relatively close to baseline levels, CI the injured/treated and injured/untreated groups declined gradually over the course of the experiments.

After an initial increase in systemic vascular resistance index (SVRI) to approximately 1 hour after the start of treatment, SVRI began to decrease in all experimental groups before plateauing after 12 hours of treatment followed by a gentle increasing trend until the end of the experiments. SVRI in the injured/treated group was consistently below that of the other groups during treatment while that of the injured/untreated group was correspondingly higher. There was no significant difference in SVRI between the groups.

Pulmonary vascular resistance index (PVRI) remained close to baseline levels for all of the groups until 1 hour after the start of treatment when that of the injured groups progressively increased while that of the uninjured groups remained lower with a subtle decrease to 6 hours post-treatment. PVRI in the placebo/untreated sheep remained near baseline levels and the lowest throughout the course of the experiment.

After a small peak corresponding to the start of treatment, right ventricular stroke work index (RVSWI) in the uninjured sheep gradually increased while that of the injured sheep decreased. There was a significant difference ($p = 0.0196$) in RVSWI gap between the uninjured/treated and injured/treated groups. RVSWI in the placebo/untreated group remained high while that of the injured/treated group was consistently the lowest.

Left ventricular stroke work index (LVSWI) gradually increased in the uninjured/treated and placebo/untreated groups, before plateauing after 12 and 18 hours of treatment, respectively, while LVSWI in the injured/untreated and injured/treated groups of sheep decreased before plateauing at 12 hours and trending upward after 18 hours of treatment. LVSWI in the placebo/untreated group remained consistently higher than in the other groups while that of the injured/treated group was consistently the lowest.

Following a decrease in the coronary perfusion pressure (CPP) from baseline in the smoke-injured sheep, there was a subsequent increase in this parameter prior to a sustained decrease at 18 hours of treatment, followed by another increase. There was a significant difference in CPP ($p = 0.0018$) between the uninjured/treated and injured/treated groups and CCP in the placebo/untreated sheep remained relatively stable after an initial, subtle increase.

There was an initial subtle decrease in arterial oxygen content (C_aO_2) from baseline in all groups before a sustained increase in the injured/untreated group, a steady level in the placebo/untreated sheep, and a sharp trough in the injured/treated and uninjured/treated groups. Following the trough, the C_aO_2 of the injured/treated group gradually returned to baseline levels while that of the uninjured/treated group continued on a downward trend. There was a significant difference ($p < 0.0085$) in C_aO_2 between the uninjured/treated and injured/treated groups.

There was a slight decrease in the oxygen delivery index (DO_2I) in all groups to 1 hour of treatment before a further marked decrease, except for the placebo/untreated sheep. There was a significant difference ($p = 0.0013$) in DO_2I between the uninjured/treated and injured/treated groups. The injured/treated group had the lowest DO_2I compared with the other groups while the placebo/untreated sheep maintained the highest DO_2I profile.

The oxygen extraction index (O_2EI) decreased in all groups before plateauing at approximately 6 hours after the start of treatment. There was a significant difference ($p = 0.0247$) in O_2EI between the injured/treated and uninjured/treated groups. O_2EI in the injured/treated and injured/untreated groups was consistently lower and higher, respectively, compared with those of the other groups.

Fluid input and urine output

The volume of intravenous fluids administered to sheep in the different experimental groups varied. The injured/treated sheep had the highest fluid requirements while the placebo/untreated sheep required the least. There was a significant difference ($p < 0.0001$) in fluid requirements between uninjured/treated and injured/treated sheep.

The injured/untreated and injured/treated groups produced the least and most urine on average, respectively. There was no significant difference in urine output between the uninjured/treated and injured/treated groups.

Anaesthetics

There was a significant difference ($p < 0.0001$) in the amount of alfaxalone required between the uninjured/treated and injured/treated groups. The uninjured/treated group required more alfaxalone on average and the injured/untreated group required the least amount on average. Ketamine requirements differed between groups, with the injured/untreated group requiring the highest

amount on average and the injured/treated group requiring the least. There was no significant difference in the quantities of ketamine required between the uninjured/treated and injured/treated groups but significant differences in midazolam requirements occurred between the uninjured/treated and injured/treated groups ($p = 0.0067$).

Anticoagulation

There were no significant differences in heparin infusion doses between the uninjured/treated and injured/treated sheep. Heparin requirements for the placebo/untreated group were the lowest. Activated clotting time increased sharply from baseline during pre-treatment and peaked 1 hour after the start of treatment before decreasing sharply and plateauing. There were no significant differences in activated clotting time between groups.

ECLS circuit observations

There were significant differences in the ECLS pump speed, blood flow, and pressure differential between the uninjured/treated and injured/treated groups. Pump speed, blood flow, and pressure differential were significantly different ($p = 0.0022$), ($p = 0.0095$) and ($p = 0.0041$), respectively, between the two groups receiving ECLS. These parameters in the uninjured/treated group were consistently higher than those of the injured/treated group.

Body temperature

Body temperature in the untreated groups gradually increased from baseline levels and plateaued at approximately 6 hours after starting treatment, and remained higher than for the treated groups. There was no significant difference in body temperature between the treated groups.

Inflammatory cells and cytokines

Data were available in abstract form on inflammatory cell infiltration into the lung tissue with a trend toward increased lung injury in the sheep that inhaled smoke, showing damage to the bronchiolar lining and infiltration of inflammatory cells [11-13].

Discussion

The results of this study agree with and confirm earlier preliminary observations that ECLS causes a decrease in pulmonary compliance over time [9]. It was expected that the injured sheep would have relatively lower SpO_2 readings compared with the other groups because of episodes of hypotension with hypoxemia, which can affect pulse oximeter function [14]. The relatively low

$etCO_2$ in the injured sheep suggested that the sheep may have hyperventilated, the causes of which were evaluated with respect to reactive oxygen species or superoxide dismutase activity by a team from the source study [15, 16].

The relatively low blood pH in the injured/treated sheep suggested that the sheep tended to metabolic acidosis as the same group of animals also had low $etCO_2$. This also means that there was no respiratory component contributing to the observed acidosis. The low pCO_2 in the uninjured/treated sheep could be a result of hyperventilation and the high pCO_2 in the injured/untreated sheep suggested that CO_2 clearance was curtailed by injury.

The treatment of the sheep contributed to lung injury by causing deterioration of pO_2. The low pO_2 translated to low partial arterial oxygen pressure/inspired oxygen fraction (PaO_2/FiO_2) ratio, which was much worse in the injured sheep. This finding showed that ECLS contributed to the deterioration of the PaO_2/FiO_2 ratio in the injured/treated group of sheep, a novel finding that was also unexpected in the primary study.

The relatively higher levels of [Hb] in the injured sheep suggested that these animals could have been dehydrated secondary to excessive fluid loss from inflammation and increased vascular permeability [17] despite intravenous fluid replacement. However, blood total protein and albumin levels, better predictors of dehydration in sheep [18], were not measured.

The inverse decrease in FO_2Hb relative to FCOHb following smoke injury was expected and agreed with other studies [17, 19, 20]. It has recently been demonstrated that FCOHb is not correlated to the degree of lung injury [17]. The gradual decrease in MetHb was probably caused by the enzymatic activity of methaemoglobin reductase [21] and the higher Hct observed in the injured sheep could have been due to dehydration because HCT was measured by an automated method. The $[Ca^{2+}]$ was lower than the published normal level of 2.4 mmol/L [22]. Stress associated with yarding of the sheep and phosphorus imbalance in feed are the most likely suggested causes of low $[Ca^{2+}]$ [23]. Fasting the sheep for 24 hours prior to the experimental procedures could also have contributed to the relatively low $[Ca^{2+}]$.

The increase in $[Cl^-]$ beyond the normal range of 105 –110 mmol/L [22] during the experiments suggests that the sheep may have developed respiratory alkalosis. Hyperventilation or metabolic acidosis resulting from sustained salivary loss of sodium bicarbonate that was more severe in the injured/treated group may have played a role hyperchloraemia, because Cl^- is known to replace HCO_3^- in the latter's loss [24, 25]. Baseline $[K^+]$ in all of the sheep was below the published normal range of 4–5 mmol/L [22] and this relative hypokalaemia may have been related to low $[K^+]$ in the diet [26-28]. The normal anion gap with decreased HCO_3^- confirmed the presence of

hyperchloraemic acidosis in all but the placebo/untreated sheep. The cause of the hyperchloraemia was likely the prolonged administration of 0.9% NaCl.

Although normal [Glu] in ruminants is usually lower than for other species, its relative progressive increase in the injured sheep may have been related to stress and severe pain associated with injury or the development of enterotoxaemia [29, 30]. The relative increase in [Lac] beyond the reported normal range of 1–2 mmol/L in the injured sheep suggested dehydration, trauma, and sepsis [31]. Sepsis, in particular, is a concern with sub-optimal rumen function leading to loss of its buffer effect and increasing numbers of anaerobic bacteria with prolonged hypomotility, such as occurs during long-duration anaesthesia. Therefore, the increases in both [Glu] and [Lac] are consistent with severe injury.

The elevated [Base (ecf)] above +2 mmol/L for most of the first 12 hours in the placebo/untreated and injured/untreated sheep suggested that the sheep were metabolically alkalotic [29] before returning to normal levels. The relatively low [Base (ecf)] (less than −2 mmol/L) was consistent with HCO_3^- loss and the tendency to metabolic acidosis [29] in the injured/treated sheep. The marked decrease in [HCO_3^-] in the injured sheep was consistent with metabolic acidosis and was more severe in the injured/treated group, suggesting that ECLS was a contributing factor.

The resting HR of sheep is 50–80 beats/min [22]. In a study that instrumented conscious sheep, the baseline heart rate was registered as 106 ± 9 beats/min [32]. In the present report, all of the sheep had a relatively high HR, suggesting that stress and pain were contributing factors. The gradual decrease in HR during the course of the experiments was consistent with the effects of anaesthesia [22].

In sheep, a mean arterial pressure below 60 mmHg indicates inadequate tissue perfusion [22]. Although the MAP values in the injured sheep were lower than for the uninjured, MAP values were still within the published normal value of 70 mmHg [22]; the magnitude of injury was again a predictor of how low the MAP was. Another predictor for the severity of the injury was the mean pulmonary artery pressure, which was highest for the injured/treated sheep. The baseline values for MPAP were higher than the 17 ± 1 mmHg reported in another study using sheep [32]. The baseline CVP in all of the sheep in the present report was > 10 mmHg, which was much higher than the 5.5 ± 1.2 mmHg reported elsewhere [32] in instrumented conscious sheep and a novel finding in this study. The severity of injury and treatment contributed to the CVP elevations in this study.

There was a benefit of ECLS treatment for SvO_2 as it remained high for both the injured/treated and uninjured treated groups. The consistently low SvO_2 in the injured/untreated group was expected because of the slightly reduced cardiac output in this group; however, this level of SvO_2 was still higher than that reported in other studies [32]. Smoke injury was associated with a sustained decrease in cardiac output in all of the sheep that were exposed to smoke. As in CCO changes, the

SV, SVI and CI all had similar profiles for the different groups, with the injured sheep having lower values. The decrease in SVRI in all of the sheep later in the experiments suggested that there was systemic vasodilation. In contrast, the increase in PVRI in the injured sheep suggested that vasoconstriction was caused by exposure to smoke injury. The exposure to smoke injury worsened both RVSWI and LVSWI while there was an increase in both parameters in the uninjured sheep. Reduced RVSWI is associated with poor functioning of the right ventricle [33, 34] and LVSWI is a reliable parameter for left ventricular function [35].

The reduction in coronary perfusion pressure in the injured/treated, and to a certain extent the uninjured/ treated sheep, suggested that ECLS contributed to the decrease in CPP, in addition to smoke injury. CPP is an indicator of myocardial perfusion and has been proposed as a drug target during resuscitation [36]. The observations in the present study support the suggestion that CPP could be used to predict the severity of injury in sheep.

The apparent increase in CaO_2 in the injured sheep could have been due to the relative increase in [Hb] secondary to dehydration. The low DO_2I in the injured/treated and uninjured/treated groups suggested that ECLS had a contribution, in addition to smoke, based on the relatively higher DO_2I in the injured/untreated sheep. Interestingly, the O_2EI had a comparable profile to that of the PaO_2/FiO_2 ratio, and could also be used to predict the contribution of ECLS to smoke-related injury.

The smoke-injured sheep required considerable amounts of intravenous fluids to compensate for the losses from pulmonary exudation and inflammation [17, 19]. The mean urine production in all groups was marginally lower than the published normal of 1.2 mL/h [22] but still considered to be within the acceptable range for this cohort of sheep. The amount of anaesthetic drugs used was considered adequate for the experiments. Heparin infusion was indicated to prolong the activated clotting time to minimise the risk of thrombosis during intravascular procedures [37].

The reduction in the ECLS pump speed, flow, and the pressure differential could have resulted from systemic hypotension contributing to low amounts of blood to the pump. The ECLS was configured such that the centrifugal pump pulled blood from the inferior vena cava and returned it into the right atrium; therefore, if the circulating volume was low, the flow would decrease for a given pump speed and in this case, both rpm and flow dropped. Centrifugal ECLS pumps are known to be preload dependent and afterload sensitive [38], making rpm and flows directly proportional to each other. The reason for the systemic hypotension remains undetermined. It is possible that an unknown pulmonary component or product produced in the smoke-damaged lungs played a role.

The body temperature of the sheep was generally within the physiological range.

Certain observations about this study could affect the interpretation of red blood cell indices and their derivatives. For instance, animals differ from humans in that estimated changes in plasma volume is preferably determined by changes in packed cell volume (PCV) or haemoglobin

concentration and total plasma protein (TPP) [39-41]. Also, in animals, there is a wider range of normal PCV than TPP [42]. In domestic animal critical care, the change in both PCV and TPP is most useful as a crude index of change in plasma volume [43]. A centrifuge that spins minute amounts of blood for rapid, cost-effective determination of PCV and TPP permits instant adjustments in the animal's fluid needs. However, measurements of PCV and TPP were not made in the primary study. As with all data that are collected with different objectives, it was considerably tedious to align certain time points with real-time observations made in the laboratory, especially for the manually input data. There was also no information about pre-anaesthetic blood tests.
An additional limitation relates to the first objective of creating a data management system for tabulating large data sets from human studies using animal models. Because the method has not been validated, it is considered preliminary and further validation studies are required. Also, the numbers of sheep were low and this was especially so in the injured/untreated and placebo/untreated groups, preventing comparisons between the treated and untreated sheep. A further limitation is that cytokine levels, as predictors of lung injury, were not quantified. Using ELISA assays to quantify cytokine levels proved difficult and the cost was prohibitive in the present study. It is partly for this reason that pioneering studies for the development of proteogenomic assays were proposed [44] as an alternative to ELISA to learn from circulating markers of acute inflammation in injured sheep used as models of intensive care, to understand critical illness.

Conclusions

The results of this study demonstrated that this preliminary method of raw data processing was effective and helped show that ECLS contributed to further worsening of pulmonary pathology by reducing lung compliance and PaO_2/FiO_2 ratio. The O_2EI changes mirrored those of the PaO_2/FiO_2 ratio, and decreasing CPP was a predictor of a greater magnitude of cardiopulmonary injury in sheep. These novel observations further understanding of similar pathology in other patients; for instance, in the resuscitation of smoke-injured animals in house or bush fires. A similar data processing approach could be used in evaluating the effectiveness of a given experimental or clinical intervention to further the understanding of the clinical condition being studied, and to aid in the formulation of treatments aimed at improving the survival of animal patients. In veterinary medicine, albeit now a considerably expensive and remote option, ECLS knowledge could complement the treatment of potentially reversible aspiration pneumonia, a secondary complication associated with both *Ixodes holocyclus* toxicity and laryngeal paralysis, in valuable companion animals.

List of abbreviations

ANOVA: Analysis of variance

C24H:Control experiment for 24 hours

CaO2:Arterial oxygen content

CCO:Continuous cardiac output

CI:Cardiac index

CO:Cardiac output

CPP:Coronary perfusion pressure

[Hb]:Haemoglobin concentration

CVP:Central venous pressure

DO2I:Oxygen delivery index

E24H:Extracorporeal life support for 24 hours

E2H:Extracorporeal life support for 24 hours

ECLS:Extracorporeal life support

ECMO:Extracorporeal membrane oxygenation

etCO2:End tidal carbon dioxide tension

FCOHb: Fraction of carboxyhaemoglobin

FiO_2:Fraction of inspired oxygen

FO_2Hb:Fraction of oxyhaemoglobin

HR:Heart rate

LVSWI:Left ventricular stroke work index

MAP:Mean arterial pressure

MERF:Medical Engineering Facility

MetHb:Methaemoglobin

MPAP:Mean pulmonary artery pressure

O2EI:Oxygen extraction index

PaO2:Arterial partial pressure of oxygen

PAP:Pulmonary artery pressure

pCO_2:Partial pressure of carbon dioxide

PCV:Packed cell volume

PEEP:Positive end-expiratory pressure

pO_2:Partial pressure of blood oxygen

PVRI:Pulmonary vascular resistance index

QUT:Queensland University of Technology

QUT-MERF:Queensland University of Technology Medical Engineering Research Facility

SC24:Smoke control experiment for 24 hours

SD:Standard Deviation

SE24H:Smoke injury and extracorporeal life support for 24 hours

SEA24H: Smoke injury, stored blood transfusion and extracorporeal life support for 24 hours

SEF24H: Smoke injury, fresh blood transfusion and extracorporeal life support for 24 hours

SPO_2:Blood oxygen saturation

SV:Stroke volume

SVI:Stroke volume index

SvO_2:Mixed venous oxygen saturation

SVR:Systemic vascular resistance

SVRI:Systemic vascular resistance index

TPP: Total plasma protein

UQ:The University of Queensland

Competing interests

A previously undisclosed conflict of interest became apparent from a section of adjunct persons within the research group when important early findings of this paper were first presented at an academic milestone seminar at The University of Queensland in August 2013. Therefore, this report comprises work completed during Research Higher Degree studies from September 2012 to 23 August 2013, only.

Author Contributions

The author (SC) was solely responsible for the study design, writing the manuscript, analysing and interpreting the data, final approval of the manuscript, and is fully accountable for the work.

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Figure Legends

Figure 1. Uncleaned manually input extra-corporeal membrane oxygenation (ECMO) treatment monitoring data in sheep

Figure 2. Cleaned and time-point annotated manually input extra-corporeal membrane oxygenation (ECMO) treatment monitoring data in sheep

Figure 3. Uncleaned electronically acquired physiological monitoring and treatment data during extra-corporeal membrane oxygenation (ECMO) in sheep

Figure 4. Cleaned electronically acquired physiological monitoring and treatment data during extra-corporeal membrane oxygenation (ECMO) in sheep

Figure 5. Data integrity checks and artefact removal of physiological monitoring and treatment data during extra-corporeal membrane oxygenation (ECMO) in sheep

Figure 6. Completed extra-corporeal membrane oxygenation (ECMO) treatment data sheet in sheep listed by parameter for further data analysis

a: treatment timeline, b: all experiments where sheep received smoke inhalation acute lung injury (SE24H), c: parameter trend plot, and d: descriptive statistics panel

Figure 7. Pulmonary compliance of smoke and non-smoke injured sheep receiving extracorporeal membrane oxygenation (ECMO) support alongside untreated controls

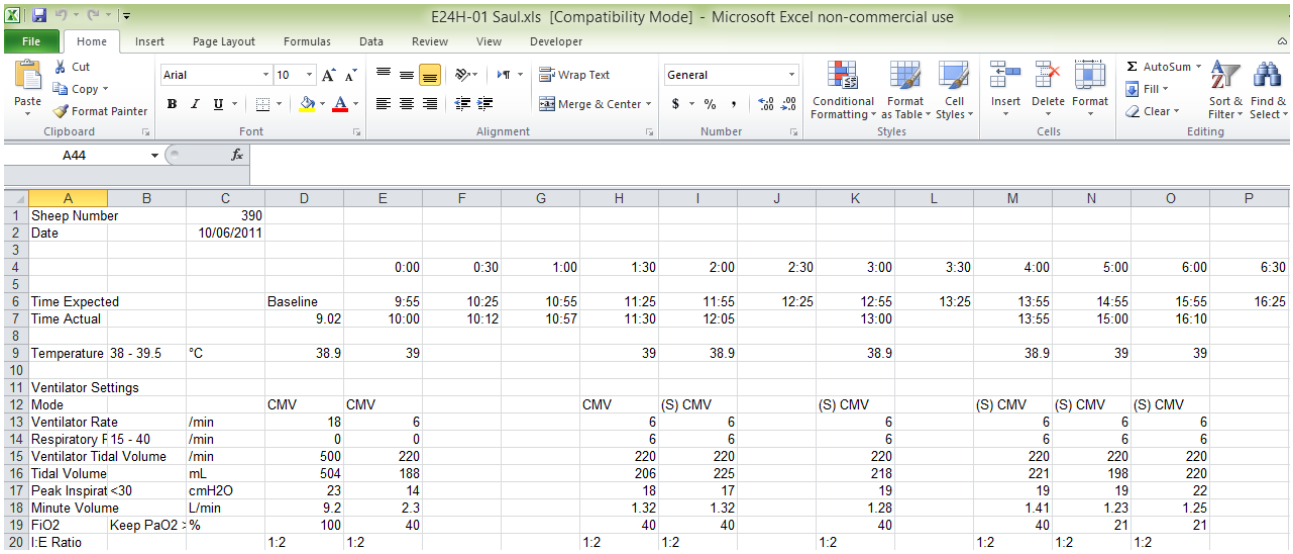
Dotted lines represent error bar margins. Values are presented as mean ± standard deviation.

Figure 8. Arterial oxygen tension for injured and uninjured sheep receiving extracorporeal membrane oxygenation (ECMO) support alongside untreated controls

Arterial oxygen tension (pO_2) values are presented as mean ± standard deviation with no error bars shown.

Figures

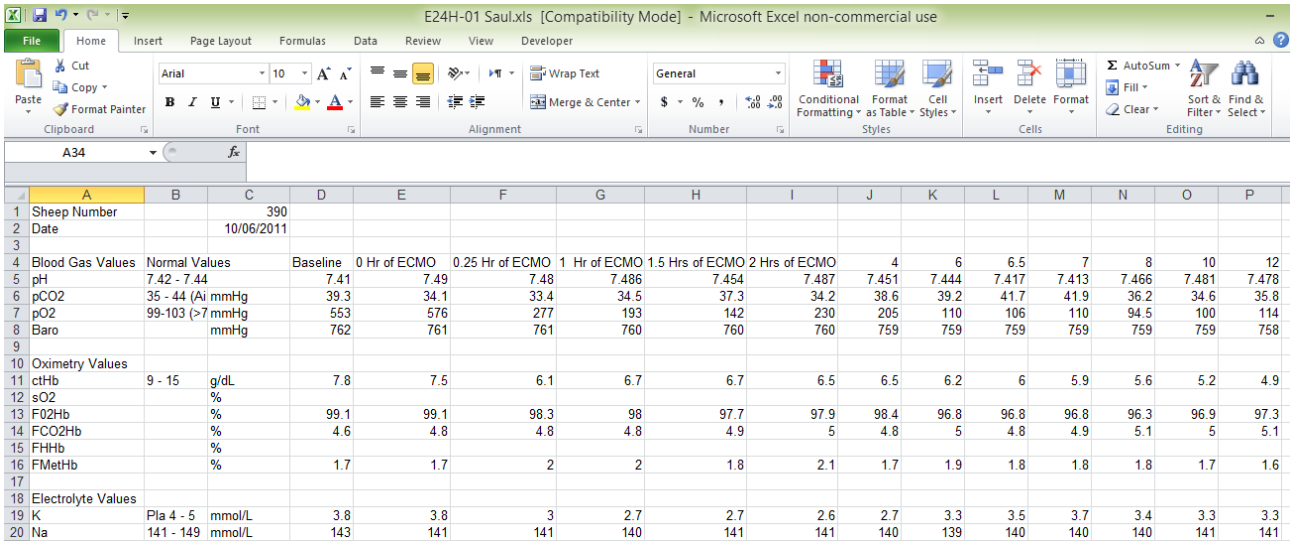
Figure 1. Uncleaned manually input extra-corporeal membrane oxygenation (ECMO) treatment monitoring data in sheep



E24H-01 Saul.xls [Compatibility Mode] - Microsoft Excel non-commercial use

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
1	Sheep Number		390													
2	Date		10/06/2011													
3																
4					0:00	0:30	1:00	1:30	2:00	2:30	3:00	3:30	4:00	5:00	6:00	6:30
5																
6	Time Expected			Baseline	9:55	10:25	10:55	11:25	11:55	12:25	12:55	13:25	13:55	14:55	15:55	16:25
7	Time Actual			9.02	10:00	10:12	10:57	11:30	12:05		13:00		13:55	15:00	16:10	
8																
9	Temperature	38 - 39.5	°C	38.9	39			39	38.9		38.9		38.9	39	39	
10																
11	Ventilator Settings															
12	Mode			CMV	CMV			CMV	(S) CMV		(S) CMV		(S) CMV	(S) CMV	(S) CMV	
13	Ventilator Rate		/min	18	6			6	6		6		6	6	6	
14	Respiratory F	15 - 40	/min	0	0			6	6		6		6	6	6	
15	Ventilator Tidal Volume		/min	500	220			220	220		220		220	220	220	
16	Tidal Volume		mL	504	188			206	225		218		221	198	220	
17	Peak Inspirat	<30	cmH2O	23	14			18	17		19		19	19	22	
18	Minute Volume		L/min	9.2	2.3			1.32	1.32		1.28		1.41	1.23	1.25	
19	FiO2	Keep PaO2 >	%	100	40			40	40		40		40	21	21	
20	I:E Ratio			1:2	1:2			1:2	1:2		1:2		1:2	1:2	1:2	

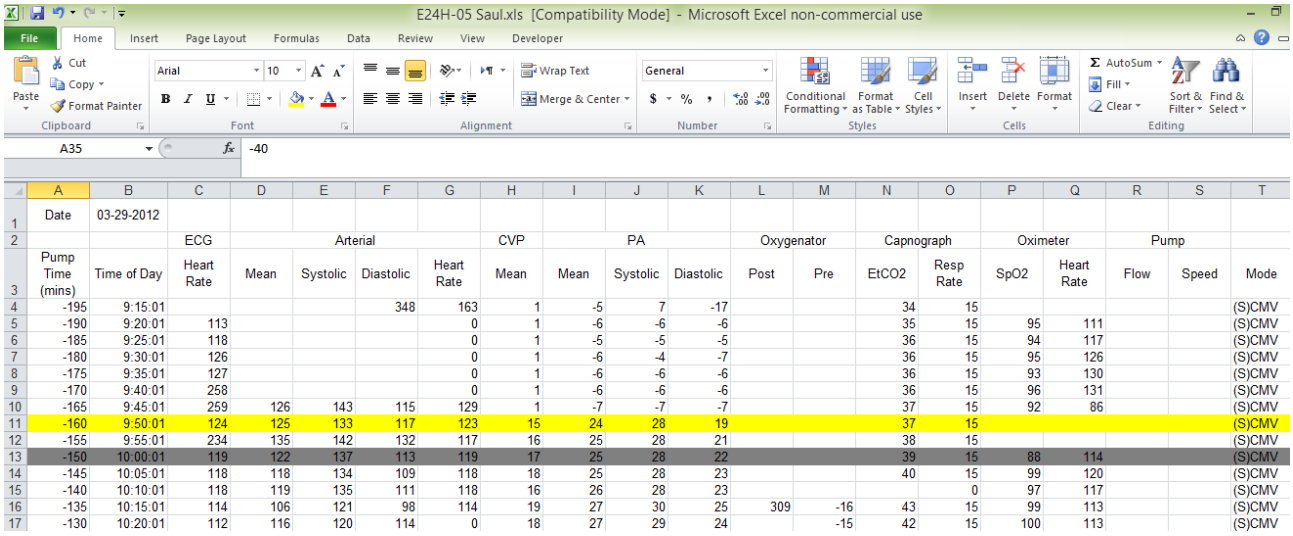
Figure 2. Cleaned and time-point annotated manually input extra-corporeal membrane oxygenation (ECMO) treatment monitoring data in sheep



E24H-01 Saul.xls [Compatibility Mode] - Microsoft Excel non-commercial use

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
1	Sheep Number		390													
2	Date		10/06/2011													
3																
4	Blood Gas Values	Normal Values		Baseline	0 Hr of ECMO	0.25 Hr of ECMO	1 Hr of ECMO	1.5 Hrs of ECMO	2 Hrs of ECMO	4	6	6.5	7	8	10	12
5	pH	7.42 - 7.44		7.41	7.49	7.48	7.486	7.454	7.487	7.451	7.444	7.417	7.413	7.466	7.481	7.478
6	pCO2	35 - 44 (Ai	mmHg	39.3	34.1	33.4	34.5	37.3	34.2	38.6	39.2	41.7	41.9	36.2	34.6	35.8
7	pO2	99-103 (>7	mmHg	553	576	277	193	142	230	205	110	106	110	94.5	100	114
8	Baro		mmHg	762	761	761	760	760	760	759	759	759	759	759	759	758
9																
10	Oximetry Values															
11	ctHb	9 - 15	g/dL	7.8	7.5	6.1	6.7	6.7	6.5	6.5	6.2	6	5.9	5.6	5.2	4.9
12	sO2		%													
13	F02Hb		%	99.1	99.1	98.3	98	97.7	97.9	98.4	96.8	96.8	96.8	96.3	96.9	97.3
14	FCO2Hb		%	4.6	4.8	4.8	4.8	4.9	5	4.8	5	4.8	4.9	5.1	5	5.1
15	FHHb		%													
16	FMetHb		%	1.7	1.7	2	2	1.8	2.1	1.7	1.9	1.8	1.8	1.8	1.7	1.6
17																
18	Electrolyte Values															
19	K	Pla 4 - 5	mmol/L	3.8	3.8	3	2.7	2.7	2.6	2.7	3.3	3.5	3.7	3.4	3.3	3.3
20	Na	141 - 149	mmol/L	143	141	141	140	141	141	140	139	140	140	140	141	141

Figure 3. Uncleaned electronically acquired physiological monitoring and treatment data during extra-corporeal membrane oxygenation (ECMO) in sheep

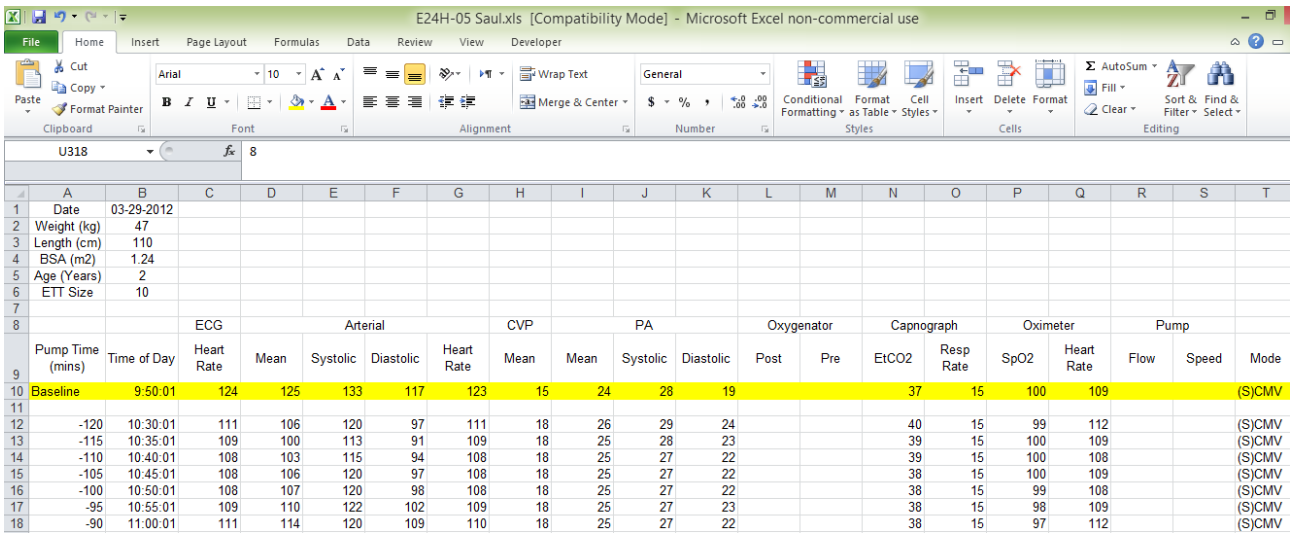


E24H-05 Saul.xls [Compatibility Mode] - Microsoft Excel non-commercial use

A35 -40

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T
1	Date	03-29-2012																		
2			ECG	Arterial				CVP	PA			Oxygenator		Capnograph		Oximeter		Pump		
3	Pump Time (mins)	Time of Day	Heart Rate	Mean	Systolic	Diastolic	Heart Rate	Mean	Mean	Systolic	Diastolic	Post	Pre	EtCO2	Resp Rate	SpO2	Heart Rate	Flow	Speed	Mode
4	-195	9:15:01				348	163	1	-5	7	-17			34	15					(S)CMV
5	-190	9:20:01	113				0	1	-6	-6	-6			35	15	95	111			(S)CMV
6	-185	9:25:01	118				0	1	-5	-5	-5			36	15	94	117			(S)CMV
7	-180	9:30:01	126				0	1	-6	-4	-7			36	15	95	126			(S)CMV
8	-175	9:35:01	127				0	1	-6	-6	-6			36	15	93	130			(S)CMV
9	-170	9:40:01	258				0	1	-6	-6	-6			36	15	96	131			(S)CMV
10	-165	9:45:01	259	126	143	115	129	1	-7	-7	-7			37	15	92	86			(S)CMV
11	-160	9:50:01	124	125	133	117	123	15	24	28	19			37	15					(S)CMV
12	-155	9:55:01	234	135	142	132	117	16	25	28	21			38	15					(S)CMV
13	-150	10:00:01	119	122	137	113	119	17	25	28	22			39	15	88	114			(S)CMV
14	-145	10:05:01	118	118	134	109	118	18	25	28	23			40	15	99	120			(S)CMV
15	-140	10:10:01	118	119	135	111	118	16	26	28	23				0	97	117			(S)CMV
16	-135	10:15:01	114	106	121	98	114	19	27	30	25	309	-16	43	15	99	113			(S)CMV
17	-130	10:20:01	112	116	120	114	0	18	27	29	24		-15	42	15	100	113			(S)CMV

Figure 4. Cleaned electronically acquired physiological monitoring and treatment data during extra-corporeal membrane oxygenation (ECMO) in sheep



E24H-05 Saul.xls [Compatibility Mode] - Microsoft Excel non-commercial use

U318 8

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T
1	Date	03-29-2012																		
2	Weight (kg)	47																		
3	Length (cm)	110																		
4	BSA (m2)	1.24																		
5	Age (Years)	2																		
6	ETT Size	10																		
7																				
8			ECG	Arterial				CVP	PA			Oxygenator		Capnograph		Oximeter		Pump		
9	Pump Time (mins)	Time of Day	Heart Rate	Mean	Systolic	Diastolic	Heart Rate	Mean	Mean	Systolic	Diastolic	Post	Pre	EtCO2	Resp Rate	SpO2	Heart Rate	Flow	Speed	Mode
10	Baseline	9:50:01	124	125	133	117	123	15	24	28	19			37	15	100	109			(S)CMV
11																				
12	-120	10:30:01	111	106	120	97	111	18	26	29	24			40	15	99	112			(S)CMV
13	-115	10:35:01	109	100	113	91	109	18	25	28	23			39	15	100	109			(S)CMV
14	-110	10:40:01	108	103	115	94	108	18	25	27	22			39	15	100	108			(S)CMV
15	-105	10:45:01	108	106	120	97	108	18	25	27	22			38	15	100	109			(S)CMV
16	-100	10:50:01	108	107	120	98	108	18	25	27	22			38	15	99	108			(S)CMV
17	-95	10:55:01	109	110	122	102	109	18	25	27	23			38	15	98	109			(S)CMV
18	-90	11:00:01	111	114	120	109	110	18	25	27	22			38	15	97	112			(S)CMV

Figure 5. Data integrity checks and artefact removal of physiological monitoring and treatment data during extra-corporeal membrane oxygenation (ECMO) in sheep

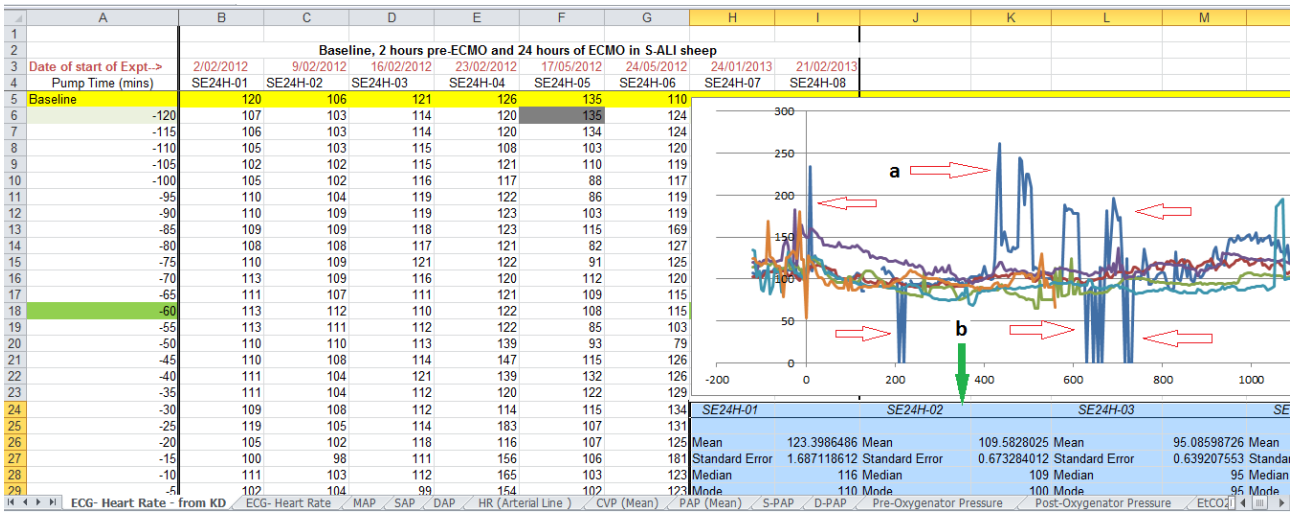


Figure 6. Completed extra-corporeal membrane oxygenation (ECMO) treatment data sheet in sheep listed by parameter for further data analysis a: treatment timeline, b: all experiments where sheep received smoke inhalation acute lung injury (SE24H), c: parameter trend plot, and d: descriptive statistics panel

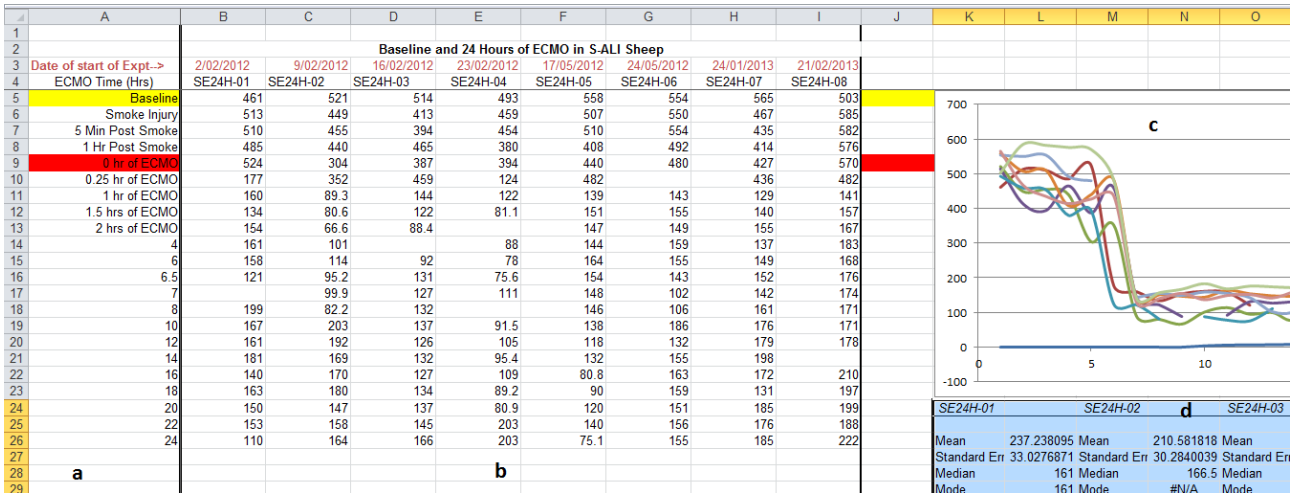


Figure 7. Pulmonary compliance of smoke and non-smoke injured sheep receiving extracorporeal membrane oxygenation (ECMO) support alongside untreated controls

Dotted lines represent error bar margins. Values are presented as mean ± standard deviation.

FIGURE 7. Pulmonary compliance (Mean ± SD) of smoke and non-smoke injured sheep receiving extracorporeal membrane oxyegenation (ECMO) support alongside untreated controls. Dotted lines represent error bar margins

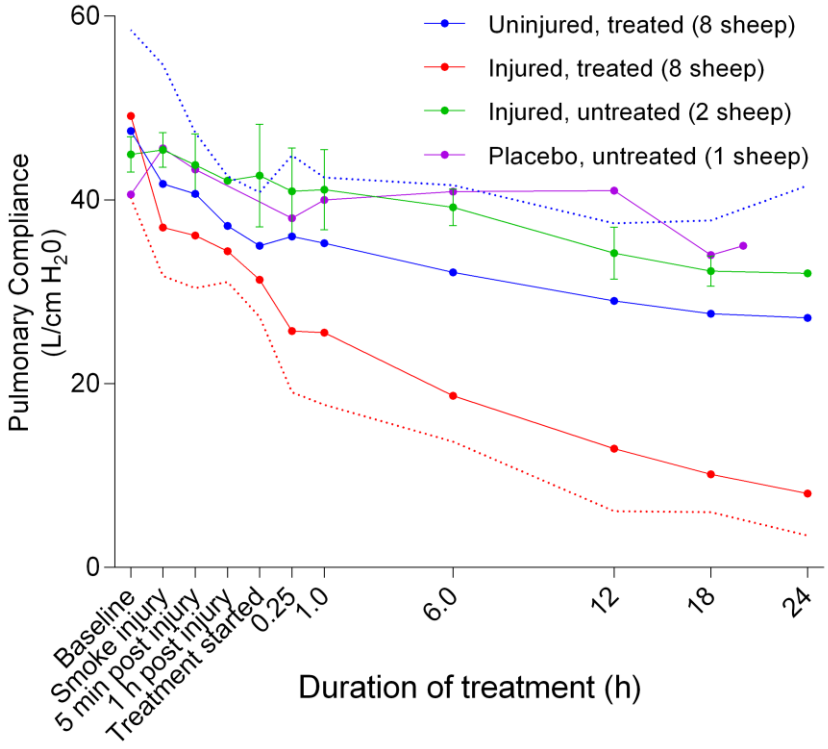
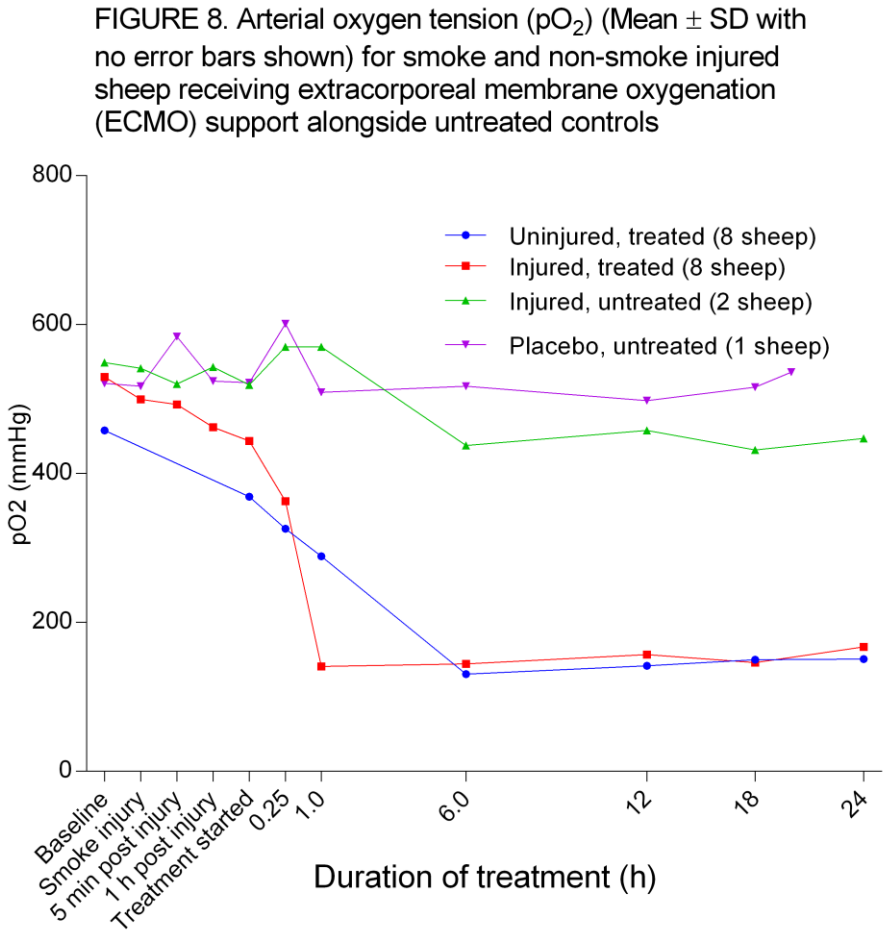


Figure 8. Arterial oxygen tension for injured and uninjured sheep receiving extracorporeal membrane oxygenation (ECMO) support alongside untreated controls

Arterial oxygen tension (pO_2) values are presented as mean ± standard deviation with no error bars shown.



Tables and captions

Table 1 Subject characteristics in treated and untreated groups (control experiments) of sheep

Experiment Group	Date	Sheep No.	Age (Y)	Weight (kg)	Length (m)	BSA
E24H	06/10/2011	E24H-01/390	2	50	110	1.29
	20/10/2011	E24H-02	2	47.6	110	1.25
	17/11/2011	E24H-03	2	51	110	1.31
	01/03/2012	E24H/4616	2	50	110	1.29
	29/03/2012	E24H-05/4627	2	47	110	1.24
	04/04/2012	E24H-06/4146	2	40	110	1.11
	12/04/2012	E24H-07/4032	2	52.5	110	1.34
	03/05/2012	E24H-08/4630	2	53	110	1.34
SE24H	02/02/2012	SE24H-01/4139	2	44	110	1.19
	09/02/2012	SE24H-02/4542	2	53	110	1.34
	16/02/2012	SE24H-03/4280	2	45.5	110	1.21
	23/02/2012	SE24H-04/4624	2	50	110	1.29
	17/05/2012	SE24H-05/4458	2	55	140	1.38
	24/05/2012	SE24H-06/8461	2	46	140	1.22
	24/01/2013	SE24H-07/09C8032	3	52	130	1.33
	21/02/2013	SE24H-09A0142	2	50	140	1.29
SC24H	18/06/2013	SC24H-01	2	51	140	1.31
	27/06/2013	SC24H-02	2	57	140	1.41
C24H	08/08/2013	C24H-01	2	53	140	1.34

Key: BSA=Body surface area; E24H=Uninjured sheep treated with extracorporeal life support (ECLS) for 24 hours (uninjured/treated); SE24H= Smoke-induced acute lung injured sheep treated with ECLS for 24 hours (injured/treated); SC24H= Smoke-induced acute lung injured sheep monitored for 24 hours without ECLS (injured/untreated); C24H=Sheep subjected to room air injury as a control for smoke and monitored for 24 hours without ECLS (placebo/untreated).

***Correspondence with journals' Executive Editor BMC Research Notes**

From: BioMed Central Editorial <researchnotes@biomedcentral.com>

Sent: 08 July 2015 02:56

To: Saul Chemonges

Subject: MS: 1245248645165367 - Learning from critical care management of sheep receiving extra-corporeal membrane oxygenation for smoke-induced acute lung injury as a tool for processing large clinical datasets

MS: 1245248645165367

Learning from critical care management of sheep receiving extra-corporeal membrane oxygenation for smoke-induced acute lung injury as a tool for processing large clinical datasets

Dear Dr Chemonges,

Firstly, please accept my apologies for the delay in making this decision on your paper. We have now been able to discuss your revised manuscript with our Editorial Board and, based on their opinions, we feel that you have fully addressed the comments of our referees. As such, in principle we would be happy to accept your manuscript for publication. However we do have some editorial concerns which we would ask you to address before we can make a final decision on your paper:

- In your Competing Interests section you state "A previously undisclosed conflict of interest became apparent from a section of adjunct persons within the research group when important early findings of this paper were first presented at an academic milestone seminar at The University of Queensland in August 2013. Therefore, this report comprises work completed during Research Higher Degree studies from September 2012 to 23 August 2013, only."

Your meaning in this section is a little unclear to us. Could you clarify more exactly what conflict of interest became apparent when you made this presentation? Was there a dispute over the use of your data from a previously existing research project? If so, could you clarify why it was considered acceptable to use data from September 2012 to August 2013, but not data from outside this timeframe?

- You clearly state in your background section that your study utilises data from an ongoing research project with a separate focus. However, it is not exactly clear which study this is or who is involved in this study. Could you be more specific about the researchers involved in this study and what published studies have already been published from it?

- In your Acknowledgements section you state that "Gratitude is extended to Dunster KR, Diab S and Platts D for considerable assistance in the animal laboratory and sharing data". Could you be a bit more specific about any permissions you required to use this data and specifically state that these researchers gave you explicit permission to use data arising from their study (if this was the case).

We would be grateful if you could address the comments in a revised manuscript and provide a cover letter giving a point-by-point response to the concerns.

Please also ensure that your revised manuscript conforms to the journal style (http://www.biomedcentral.com/bmcresnotes/ifora/). It is important that your files are correctly formatted.

We look forward to receiving your revised manuscript by 4 August 2015. If you imagine that it will take longer to prepare please give us some estimate of when we can expect it.

You should upload your cover letter and revised manuscript through http://www.biomedcentral.com/manuscript/login/man.asp?txt_nav=man&txt_man_id=1245248645165367. You will find more detailed instructions at the base of this email.

Please don't hesitate to contact me if you have any problems or questions regarding your manuscript.

With best wishes,

Dr Christopher Foote
Executive Editor
Tel: +44 (0) 20 3192 2013
e-mail: researchnotes@biomedcentral.com
Web: http://www.biomedcentral.com/

From: Saul Chemonges
Sent: 24 July 2015 23:30
To: BioMed Central Editorial
Subject: Re: 1245248645165367 - Learning from critical care management of sheep receiving extra-corporeal membrane oxygenation for smoke-induced acute lung injury as a tool for processing large clinical datasets

Dear Dr Christopher Foote,

I addressed the comments in a revised manuscript and provided a cover letter giving a point-by-point response to the editorial concerns and resubmitted the manuscript on 7th July 2015.
When I logged in the tracking system today, there's a message that reads "Please go to https://www.editorialmanager.com/resn to track and manage those manuscripts." I was wondering if I should have made the resubmission through the new editorial manager instead.

Kind regards,
Saul Chemonges

From: Foote, Christopher <Christopher.Foote@biomedcentral.com>
Sent: 07 November 2015 02:32
To: Saul Chemonges
Subject: Your submission to BMC Research Notes

MS: 1245248645165367
Research article
Learning from critical care management of sheep receiving extra-corporeal membrane oxygenation for smoke-induced acute lung injury as a tool for processing large clinical datasets
Saul Chemonges
BMC Research Notes

Dear Dr Chemonges,

I am writing to you regarding your submission (above) to *BMC Research Notes*. Thank you for responding to our previous request for clarification over the source of the data used in your paper. My apologies for how long it has taken us to respond.

We do still have some further clarifications we hope you can provide. Would you be able to answer the following questions:

- Can you confirm that this manuscript reports data collected during a year that you spent working with John Fraser in his lab?

- Can you confirm that it is correct that you are the only person who qualifies for authorship on this paper? *BMC Research Notes* follows the ICMJE guidelines for

authorship, which I paste below. Based on these guidelines can you confirm that you are the only person who meets these criteria for authorship on this paper?

An 'author' is generally considered to be someone who has made substantive intellectual contributions to a published study. According to the International Committee of Medical Journal Editors (ICMJE) guidelines, to qualify as an author one should have:

1. made substantial contributions to conception and design, or acquisition of data, or analysis and interpretation of data;
2. been involved in drafting the manuscript or revising it critically for important intellectual content;
3. given final approval of the version to be published. Each author should have participated sufficiently in the work to take public responsibility for appropriate portions of the content; and
4. agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Acquisition of funding, collection of data, or general supervision of the research group, alone, does not usually justify authorship.

- Can you confirm that you have all the necessary permissions to use and publish the data contained in your manuscript?

I hope you do not object to providing this further clarification on these issues. I am sure you can appreciate it is important for us to be sure of these issues before we publish a paper. As part of this, we also plan to contact John Fraser to ask him to confirm that he is happy for you to utilise and publish this data collected in his lab and that he agrees you should be the only author on the paper.

I look forward to hearing from you.

Best wishes,
Dr Christopher Foote

Executive Editor
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London WC1X 8HB

Tel: +44 (0) 20 3192 2000
e-mail: christopher.foote@biomedcentral.com
Web: www.biomedcentral.com/authors/bmcseries#journallist

From: Foote, Christopher <Christopher.Foote@biomedcentral.com>
Sent: 12 November 2015 20:45
To: Saul Chemonges
Subject: RE: Your submission to BMC Research Notes
Dear Dr Chemonges,

I am just writing to follow up on my email from last week (below) to confirm that you received it and will be able to provide us with the requested clarifications regarding your study.

I look forward to hearing from you.

Best wishes,
Dr Christopher Foote

Executive Editor
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Web: www.biomedcentral.com/authors/bmcseries#journallist

From: Saul Chemonges <s.chemonges@uq.edu.au>
To: John Fraser <j.fraser@uq.edu.au>
Cc: "Christopher.Foote@biomedcentral.com" <Christopher.Foote@biomedcentral.com>; Saul Chemonges <s_chemonges@yahoo.com>; UQ Graduate School Dean <dean@gradschool.uq.edu.au>
Sent: Saturday, 30 April 2016, 14:46
Subject: Fw: Your submission to BMC Research Notes

Dear Prof John Fraser,

I submitted the attached manuscript to BMC Research Notes a while back from work in my thesis that was conducted at CCRG Lab at TPCH. The Executive Editor (Dr Christopher Foot) has requested for further clarifications regarding the manuscript as detailed below.

To reply to the clarifications, I intend to maintain affirmative answers to the questions raised by the Editor. I believe I have made the appropriate acknowledgements in the attached copy of the manuscript. Please kindly let me know if you have any concerns before I reply to Dr Foote.

Kind regards,

Saul Chemonges

From: Saul Chemonges
Sent: 07 May 2016 18:05
To: Foote, Christopher
Subject: Re: Your submission to BMC Research Notes

Dear Dr Foote,

My apologies for the delay in getting back to you regarding my article.

I only recently returned from an unscheduled extended leave of absence.

As you may have noticed, I have written to Prof Fraser regarding the clarifications that I copied to you via my other email address. Hopefully we will hear from him soon.

Many thanks,

Saul Chemonges