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Citation for published version:

Digital Object Identifier (DOI):
10.1016/j.vaa.2019.04.004

Link:
Link to publication record in Edinburgh Research Explorer

Document Version:
Peer reviewed version

Published In:
Veterinary Anaesthesia and Analgesia

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Transcranial Bioimpedance Measurement in Horses: a pilot study

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Authors’ Contributions: RG: study design, data collection, interpretation of data and writing the manuscript. MS: interpretation of data, study design and statistical analysis. IP: interpretation of data and study design. REC: study design, interpretation of data and reviewing the manuscript.

Conflict of Interest: The authors declare no conflict of interest.

Funding: This research did not receive any grant from funding agencies in the public, commercial, or not-for-profit sectors.

Running Head: Transcranial bioimpedance in horses.
Abstract

Objective This pilot study aimed to evaluate feasibility of transcranial bioimpedance (TCBI) measurement and variability of TCBI values in healthy conscious horses and to study effects of body position and time on TCBI in anaesthetised horses.

Study Design Prospective observational study.

Animals Four research horses and sixteen client-owned horses presented for surgery.

Methods After establishing optimal electrode position using computed tomography (CT) scans of cadaver heads, TCBI [described using impedance at zero frequency, R₀ (Ω)] was measured in four conscious, resting horses to investigate feasibility and changes in TCBI over time (80 minutes). Data were compared using a paired t-test. TCBI was then measured throughout anaesthesia (duration 92 ± 28 minutes) in 16 horses in dorsal and lateral recumbancy. Data were analysed using a general linear model (GLM); gamma regression was chosen as a model of characteristic impedance [Z_c; (Ω)] against time. Data are presented as mean ± standard deviation.

Results No change in R₀ was seen in conscious horses (age = 15.3 ± 7.3 years, body mass = 512 ± 38 kg) over 80 minutes. The technique was well-tolerated and caused no apparent adverse effects. In 16 horses (age = 7.4 ± 4.7 years; body mass = 479 ± 134 kg) anaesthetised for 92 ± 28 minutes, Z_c fell during anaesthesia, decreasing more in horses in lateral recumbancy when compared to horses in dorsal recumbancy (p = 0.008). There was no relationship between Z_c and body-mass or age.
Conclusions and clinical relevance TCBI is readily measured in horses. TCBI did not change with time in conscious horses, but decreased with time in anaesthetised horses; this change was greater in horses in lateral recumbancy. This indicates that changes in TCBI in anaesthetized horses may be related to the effects of recumbancy, general anaesthesia, surgery or a combination of these factors.

Keywords general anaesthesia, horse, bioimpedance, transcranial bioimpedance.

Introduction

Bioimpedance refers to the resistance measured to alternating current flow through living tissue (Shaw et al. 2012) and has been used to study body composition (Cornish et al. 1993) and acute fluid shifts (Bordelon & Wingfield 2002). Bioimpedance is measured in people using commercial devices, such as the Impedimed SBF7, which do so by measuring the impedance (Ω) recorded from the tissues lying between electrodes. The recorded impedance depends on the current pathway and the applied frequency. At low frequencies, current flows predominantly through the extracellular space, but at higher frequencies, current also passes through cell membranes and so through the intracellular space (Bordelon & Wingfield 2002). The resistance (the opposition to current flow produced by the tissue pathway) and the reactance (the resistance offered to alternating current by capacitors - in this case, the cell membrane) are then plotted against each other and mathematically extrapolated. This allows the values of resistance to be calculated for infinite ($R_\infty$) and zero frequencies ($R_0$) from which the tissue’s characteristic impedance ($Z_c$) may be estimated (Shaw et al. 2012).
Multi-frequency bioimpedance analysis has been used in euhydrated horses to predict body fluid composition (Forro et al. 2000) and to estimate fluid shifts in horses subjected to dehydration and acute blood loss (Fielding et al. 2007). Cerebral impedance measurements have been used to describe intracellular fluid shifts following hypoxic insults in foetal sheep (Williams et al. 1991). When used in piglets to quantify hypoxia-induced cerebral oedema (Lingwood et al. 2002) increases in TCBI correlated with directly measured intracranial pressure (ICP). Strong correlation was also found between directly measured ICP and TCBI in anaesthetised sheep over a range of blood and intracranial pressures (Shaw et al. 2012).

Horses undergo alterations in intracranial homeostasis related to general anaesthesia such as nasal oedema (Lukasik et al. 1997). Neurological signs following anaesthesia, causing postoperative complications, have been reported (Spadavecchia et al. 2001; McKay et al. 2002). Mortality in healthy horses undergoing general anaesthesia is greater than that of other species, ranging from 0.24% (Bidwell et al. 2007) to 0.9% (Johnston et al. 2002) within 7 days of surgery. This increases to between 1.6% (Dugdale et al. 2016) and 5% (Johnston et al. 2002) when systemically ill horses are studied.

Despite the effective measurement of total body water in horses using bioimpedance technology (Forro et al. 2000; Fielding et al. 2007), and the use of TCBI to reflect changes in intracranial homeostasis in other species (Lingwood et al. 2003; Shaw et al. 2012) the use of TCBI has not been reported in horses. The objective of this pilot study was to investigate the feasibility of TCBI measurement and the quality of the resulting signal in conscious horses. TCBI would then be measured in anaesthetised horses to identify potential relationships with body position and/or duration of anaesthesia. It was
hypothesized that prolonged anaesthesia, and dorsal (rather than lateral) recumbancy
would be associated with the greatest rises in TCBI, caused by alterations in
homeostasis within the calvarium.

Materials and Methods

Ethical approval

The study was approved by the Veterinary Ethical Review Committee of the Royal
(Dick) School of Veterinary Studies, Edinburgh. The Committee did not consider that
TCBI measurement was a regulated procedure under the Animals (Scientific
Procedures) Act, 1986 because its use is non-invasive and painless, and it has been used
successfully to measure total body water in conscious horses (Forro et al. 2000).

Cadaver computerised tomography study

Computerised tomography (CT) studies of two cadaveric horses’ skulls (primarily
utilised for an unrelated dental study) were examined to determine sites for electrode
placement that would: 1) provide a current path through the calvarium which
maximized the intra:extra-calvarial distance ratio (so maximizing the former’s
representation in the overall signal), and 2) be readily identifiable using external
landmarks (to ensure consistent and accurate electrode positioning between horses).

Feasibility study

Initial studies to evaluate the feasibility and variability of TCBI in conscious horses
were undertaken. An Impedimed SBF7 device (Impedimed Limited, CA, USA) was
used to measure TCBI in four conscious, un-sedated horses belonging to the Royal (Dick) School of Veterinary Studies; details of these horses are recorded in Table 1.

Proprietary electrocardiogram (ECG) electrodes (“BlueSensor”; Ambu, Denmark) were placed in pairs on brushed clean (but unclipped) skin on both sides of the head and the four leads connected in the order: red; yellow; blue; black running from caudolateral to caudolateral; that is, a clockwise direction, as shown in Figure 1. The red and black leads deliver the current through the tissue section while the blue and yellow leads are required for bioimpedance measurement. Measurements were taken at 20-minute intervals for 80 minutes, and the horses’ heads were held in a standard, neutral position, neither raised nor lowered, while measurements were taken. At each time point, the Impedimed SBF7 directly measured impedance, resistance and reactance. From these, impedance at zero resistance ($R_0$) and impedance at infinite resistance ($R_\infty$) were estimated by the Impedimed device. Characteristic impedance ($Z_c$) was estimated by analysis of the subsequent Cole-Cole plot. These measurements were repeated 20 times at each time point (from which an average value was calculated), and the Impedimed SBF7 automatically recorded all measurements for subsequent review.

**Study of anesthetised horses**

The TCBI was measured in 16 horses anaesthetised for surgery at the Royal (Dick) School of Veterinary Studies Equine Hospital. All well-handled, client-owned horses undergoing elective procedures when the primary investigator was available were included; no exclusion criteria were applied. A sample size calculation was not performed because this was a pilot study which aimed to characterise the variability in TCBI which could be expected in anaesthetised horses. Informed owner consent was obtained. Anaesthetic technique was pre-anaesthetic medication with romifidine (100
µg kg\(^{-1}\): Sedivet; Boehringer Ingelheim, UK) or xylazine (1.1 mg kg\(^{-1}\): Channel
Chanelle Animal Health, UK), induction with ketamine (2.2 mg kg\(^{-1}\): Vetalar V; Pharmacia & Upjohn Animal Health, UK) and diazepam (0.05 mg kg\(^{-1}\): Diazepam Injection BP; Hameln Pharmaceuticals Ltd, UK) maintenance with sevoflurane (Sevoflo; Abbot Animal Health, UK) vaporized in oxygen administered via a combined large animal circle system/ventilator (Tafonius; Vetronics, UK). Morphine (0.1-0.3 mg kg\(^{-1}\): Mor
Morphine Sulphate Injection BP; Martindale Pharmaceuticals, UK) and flunixin administered as analgesics and Ringer’s lactate solution was infused at approximately 5 mL kg\(^{-1}\) hour\(^{-1}\). A multi-channel patient monitor (Datex-Engstrom S/5 Compact; Datex Engstrom Inc., MA, USA) was used to monitor a base-apex electrocardiogram, end-tidal CO\(_2\) and sevoflurane concentrations (P\(_E\) CO\(_2\) and F\(_E\) Sevo, respectively) and direct arterial blood pressure (after placement of a 22-gauge cannula in the facial, auricular or metatarsal artery). Dobutamine (1-10 µg kg\(^{-1}\) minute\(^{-1}\): Dobutamine Concentrate 250mg in 20mL; Hameln Pharmaceuticals, UK) or an ephedrine bolus (15-30 mg: Ephedrine; Martin
d Martin, UK) were administered if hypotension developed (mean arterial blood pressure < 60 mmHg). Mechanical ventilation was imposed if 1) it was required to maintain normocapnia or 2) at the anaesthetist’s discretion. Horses positioned in lateral recumbancy were positioned flat on a horizontal table; no effort was made to elevate the head relative to the rest of the body. When horses were positioned in dorsal recumbancy, the neck was not raised relative to the rest of the body, but the atlanto-occipital joint was flexed into a neutral position (with an angle of approximately 120\(^0\) between the head and neck). All horses were allowed to recover from anaesthesia without intervention.
Using the same technique as described for the initial feasibility study, $R_0$, $R_\infty$ and $Z_c$ were measured using the Impedimed SBF7: 1) after pre-anaesthetic sedation but less than 30 minutes before induction; 2) within 10 minutes after induction; and 3) at 10-minute intervals throughout anaesthesia. Twenty measurements were recorded at each time point.

**Statistics**

Data from the feasibility study were examined visually and using descriptive statistics to determine normality before being analysed using a paired t-test (Excel 2013; Microsoft, WA, USA) to compare $R_0$ at the first ($t = 0$) and final ($t = 80$) time points.

The raw data from anaesthetised horses were averaged (once per recorded time point per subject) and the characteristic impedance ($Z_c$) chosen as the variable of interest because it combined information from both resistance and reactance, and had less overall variance than the other covariates ($R_0$ or $R_\infty$). $Z_c$ was subsequently found to follow a gamma distribution and so a generalised linear modelling (GLM) approach was applied to the main data group. Initial investigation of how the main covariates varied with $Z_c$ was carried out visually; there was a strong relationship with change in $Z_c$ and time, a weaker relationship with position, sex and breed, and little to no relationship with age or body mass. The breed, mass and age covariates were not used in order that the explanatory power of the other covariates (time, position during anaesthesia) could be maximized. Similarly, the left and right lateral position covariates were combined, giving only “dorsal” or “lateral” as positional states.

A $p$-value $< 0.05$ was considered significant.
Results

Cadaver computerised tomography study

Examination of the cadaveric head CT scans revealed sites caudal to the zygomatic arch and immediately below the ear met criteria for electrode placement; that is, electrode positioning which maximized the intra-extra-calvarial distance ratio (so maximizing the former’s representation in the overall signal) and provided readily identifiable using external landmarks, allowing easily repeatable electrode placement in all horses (Figure 2).

Feasibility study

In four conscious, standing horses (demographic details and body mass are detailed in Table 1), the TBCI values as represented by $R_0$ did not change significantly with time ($p = 0.587$).

Study of anaesthetised horses

Details of age, sex, breed, body mass, procedure performed, positioning during anaesthesia and duration of anaesthesia are presented in Table 2. Gamma regression estimates are used to give the relative change of the predicted $Z_c$ value for a one unit increase in a given covariate, i.e., time, lateral position, when all other covariates are held at a nominal level. As this yields a relative, rather than an absolute, value the covariate estimates should be interpreted multiplicatively, and not additively as is common in standard linear regression. During each minute of time elapsing, $Z_c$ decreased by 0.18% compared to the previous value for all animals (Figure 3) and for
animals in lateral recumbancy $Z_c$ decreased by 7.26 % per minute. The estimated relative change was statistically significant ($p < 0.001$ for time, and $p = 0.008$ for animals in lateral position). There were increases in $Z_c$ over time relative to the predicted characteristic values in mares (31.36 %), geldings (38.51 %) and intact males (76.01 %). That is, $Z_c$ did not fall as far as the predicted value anticipated; there was no absolute rise in $Z_c$ at any particular time point. No relationship was found between change in TCBI described by $Z_c$, and the horses’ age, breed or body mass.

The measurement of TCBI was well tolerated in both conscious and anaesthetised horses; the technique was feasible and straightforward. No horse suffered from any known postoperative complication associated with anaesthesia. The thick hair coats of some horses limited electrode contact, and the adhesive gel electrodes became detached in some animals when sweating occurred during anaesthesia, requiring repositioning of the of the electrodes during the experiment.

**Discussion**

Transcranial bioimpedance measurement in horses proved to be technically easy to perform, and was well tolerated in conscious horses. Measured values for TCBI changed with time in anaesthetised horses, but not in conscious, standing animals over a similar sampling period. This indicates that changes in TCBI in anaesthetised horses could be related to the effects of recumbency, general anaesthetics, surgery or a combination of these factors.
The TCBI decreased with time in anaesthetised horses. Decreased TCBI is caused by an increase in fluid in the extracellular fluid space which facilitates current flow through sampled tissue (there being less resistance in the extracellular path). Explanations for an increased extracellular fluid space include: intravenous crystalloid fluid infusion, hydrostatic oedema, and increased capillary permeability. The administration of intravenous fluids at flows of 5-30 mL kg$^{-1}$ hour$^{-1}$ is common practice in horses, with final rates depending on concurrent losses and requirements for cardiovascular support (Hubbell 2007). Isotonic crystalloids are commonly used and they expand the extracellular fluid space after redistribution from the vascular space. This increased extracellular fluid volume could cause a progressive reduction in TCBI during anaesthesia, in line with our findings. All anaesthetised horses in this study received approximately 5 mL kg$^{-1}$ hour$^{-1}$ of isotonic fluids administered via a jugular venous cannula.

Craniofacial hydrostatic oedema is well recognised in anaesthetised horses and is caused by a combination of increased hydrostatic pressure in the nasal mucosal vascular bed and reduced venous drainage when horses are positioned in dorsal recumbancy (Lukasik et al. 1997). This effect may be compounded by the administration of volatile anaesthetics causing vasodilatation in these tissues (Lukasik et al. 1997). Horses positioned in dorsal recumbency, in which the head lies below the level of the thoracic inlet, have the largest hydrostatic gradient, with the arterial blood pressure at the circle of Willis calculated as being 15 mmHg higher than the carotid mean arterial blood pressure (Brosnan et al. 2008). Under these circumstances, increased extracellular fluid could increase intracranial volume if the intracellular and/or vascular fluid component do not contract reciprocally. The reduced venous return demonstrated by nasal oedema
suggests this re-distribution does not occur to the extent required to maintain intra- and extra- cellular fluid distribution. These peri-anaesthetic changes in intracranial fluid homeostasis, causing increases in extracellular fluid, could also contribute to the reduction in TCBI seen in anaesthetised horses in this study.

TCBI fell with time in all anesthetised horses, but there was a greater decrease in TCBI in horses anaesthetised in lateral recumbancy compared with dorsal recumbancy. This was unexpected. The vertical distance between the head and heart is greater when horses are placed in dorsal recumbancy and greater increases in extracellular fluid would be expected, although this distance was not measured in the horses studied here. However, other changes in intracranial physiology could explain the smaller than expected fall in TCBI in horses positioned in dorsal recumbancy. Hypoxic/ischaemic cerebral damage has been documented following anaesthesia in horses positioned in dorsal recumbancy (Spadavecchia et al. 2001; McKay et al. 2002). Cerebral hypoxia prevents aerobic metabolism and thus causes failure of the energy-dependant sodium-potassium pump and loss of osmoregulation. Fluid tends to shift intracellularly, with the reduced extracellular fluid volume causing an increase in TCBI (Lingwood et al. 2002). When anaesthetised piglets were subject to a hypoxic insult, TCBI was observed to rise (Lingwood et al. 2003). In horses positioned in dorsal recumbancy, hypoxic insult (causing an increase in intracellular fluid and thus a decrease in TCBI), could combine with the opposing effects of hydrostatic oedema (which increases extracellular fluid and decreases TCBI) so producing a less marked overall effect on TCBI.

Sweating could also have influence the greater decrease in TCBI measurements in horses in lateral recumbancy. Sweating could have improved the electrical contact
between the electrode pads and the horses’ heads. This effect may have been particularly pronounced at the dependant electrode on horses in lateral recumbancy because 1) the dependent side could have been warmer, and therefore sweat more, due to the insulation provided by the padded surgical table and the lack of air circulation, and 2) the weight of the head compressing the electrode between the table and the head could have caused for improved electrode contact. These conditions in horses positioned in lateral recumbancy could potentially lead to improved electrical contact and therefore less resistance in the circuit; this could have contributed to lower TCBI measurements in these animals.

TCBI can vary directly with raised ICP when caused by hypoxia (Lingwood et al. 2002), but TCBI varies indirectly with ICP when ICP is influenced by either iatrogenic hypertension or increased intracellular volume (Shaw et al. 2012). ICP was not measured in the current study; however, as intracranial pressure can reach values of 55 mmHg in horses under general anaesthesia positioned with the head down (Brosnan et al. 2008), the effect of altered ICP on TCBI in anaesthetised horses has to be considered.

Previously identified relationships between TCBI and other variables do not appear to translate to clinical situations in humans. Although TCBI changes could be detected in babies that suffered intra-partum hypoxia, increased TCBI could not be used to predict neurological outcome (Lingwood et al. 2009). In a small trial involving 10 patients, no significant relationship could be found between TCBI and invasively measured ICP (Hawthorne et al. 2018). Lingwood and colleagues considered a baseline measurement to be important in assessing TCBI changes in piglets undergoing a hypoxic insult (Lingwood et al. 2003). Baseline pre-anaesthetic TCBI measurements were taken in this
study, but the data were included in the larger dataset to simplify analysis. However, in future studies baseline measurements could be compared with changes in TCBI under general anaesthesia. TCBI values cannot be directly compared between subjects because the length of the current pathway through sampled tissue has a major effect on TCBI values (Shaw et al. 2012). This was why changes in TCBI per unit time were examined in the current study.

There were changes in TCBI over time when sex was considered and although these results were statistically significant ($p < 0.001$) they were considered to be influenced by the heavily skewed nature of the data set; half of the horses (8) were males positioned in dorsal recumbancy, and there were only five mares included in the study. Furthermore, the male animals were separated into intact males and geldings for analysis, which complicated the interpretation of the results.

There were some limitations. The dataset of conscious horses, which was limited by animal availability, was too small to confidently describe the data as being normally distributed. The dataset of anaesthetised horses was unbalanced regarding sex; although significant results were obtained regarding change in TCBI over time and sex, these have to be interpreted with caution. Some variables (breed, age, body mass, surgery performed) were omitted from the final analysis in order to give a more parsimonious model; this simplified the results but may have reduced the overall accuracy of the model predictions. All of the covariates were assumed to be linear so any non-linearities of effect will have been missed. Also, no effort was made to standardise anaesthesia, in terms of fluid administration or positioning (particularly regarding the head-heart vertical distance and the head position). The mean arterial blood pressure of the anaesthetised horses was maintained within a physiological range and the possible
effect of variation in blood pressure was not investigated in this pilot study. Variations in electrode-skin contact caused by sweating under the electrode pads could have accounted for changes in the recorded TCBI measurements; more adhesive electrode patches or needle electrodes might produce more consistent signals. Close-clipping (and perhaps shaving) the skin at electrode attachment sites may also improve contact. However, clipping was not performed in client-owned horses. These limitations could be overcome in future studies involving a standardised enrolment and anaesthetic protocol, more detailed data collection, and more complex analysis. Ideally, TCBI would be compared to direct ICP measurement; however, this invasive additional monitoring was not an option in conscious or client-owned animals.

Conclusion

In this study, we detected statistically significant changes in TCBI relative to both time and positioning during general anaesthesia in horses, and discovered no adverse effects related to the technique. The physiological alterations caused by general anaesthesia in a clinical situation complicated the interpretation of TCBI values in anaesthetised horses. Further investigation of changes in TCBI in anaesthetised horses under more controlled conditions with additional monitoring, e.g., invasive ICP measurement may provide useful information to inform management of horses in the peri-anaesthetic, recovery and immediate postoperative period.

References


Figure Legends

Figure 1 Placement of electrodes for transcranial bioimpedance (TCBI) measurement in horses, with description of leads connected to each electrode.

Figure 2 Transverse computed tomography (CT) image of a cadaveric horse skull at the level of the temporomandibular joint: white arrows indicate placement sites for transcranial bioimpedance (TCBI) electrodes.

Figure 3 Characteristic impedance \[Z_c \text{ (ohms)}\] against time (minutes) from the start of surgery in sevoflurane anaesthetised horses. The period of the study which the measurements were taken is shown by the shape of the points (pre-induction: hollow circles; during anaesthesia: solid squares) with an additional loess smoothed fit (shaded area) to indicate the overall relationship.
Table 1. Demographic details and body mass of four conscious horses from which transcranial bioimpedance (TCBI) measurements were taken.

<table>
<thead>
<tr>
<th>Number</th>
<th>Age (years)</th>
<th>Sex</th>
<th>Breed</th>
<th>Body Mass (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7</td>
<td>Gelding</td>
<td>Arab Cross</td>
<td>500</td>
</tr>
<tr>
<td>2</td>
<td>19</td>
<td>Gelding</td>
<td>Warmblood</td>
<td>550</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>Mare</td>
<td>Cob Cross</td>
<td>525</td>
</tr>
<tr>
<td>4</td>
<td>25</td>
<td>Mare</td>
<td>Welsh Cross</td>
<td>450</td>
</tr>
</tbody>
</table>
Table 2. Demographic details, body mass, duration of anaesthesia and operation details of sixteen horses from which transcranial bioimpedance (TCBI) measurements were taken in the peri-anaesthetic period.

<table>
<thead>
<tr>
<th>Number</th>
<th>Age (years)</th>
<th>Sex</th>
<th>Breed</th>
<th>Body Mass (kg)</th>
<th>Positioning during anaesthesia.</th>
<th>Surgery performed</th>
<th>Duration of anaesthesia (mins)</th>
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<tr>
<td>1</td>
<td>8</td>
<td>Mare</td>
<td>Sport Horse</td>
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<td>Dorsal</td>
<td>Tie back and ventriculectomy</td>
<td>85</td>
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<td>2</td>
<td>6</td>
<td>Gelding</td>
<td>Irish draught</td>
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<td>Right Lateral</td>
<td>Bilateral stifie arthroscopy</td>
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<td>Thoroughbred Cross</td>
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<td>Eriskay</td>
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<tr>
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<td>Castration</td>
<td>63</td>
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<td>4</td>
<td>Gelding</td>
<td>Highland Pony</td>
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<td>Castration</td>
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<td>7</td>
<td>1</td>
<td>Mare</td>
<td>Warmblood</td>
<td>450</td>
<td>Dorsal</td>
<td>Medication of hock joints</td>
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<td>Removal of splint bone fragment (left hind)</td>
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<td>Arab Cross</td>
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<td>Dorsal</td>
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<td>Arab Cross</td>
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<td>Right Lateral</td>
<td>Removal of splint bone fragment (right hind)</td>
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<td>Pony</td>
<td>364</td>
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<td>Cob</td>
<td>520</td>
<td>Right Lateral</td>
<td>Palmar desmotomy (left hind)</td>
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<td>Highland Pony</td>
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<td>Dorsal</td>
<td>Arthroscopy (right hind)</td>
<td>100</td>
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