

This is the peer-reviewed, manuscript version of an article published in *Veterinary Anaesthesia and Analgesia*. The version of record is available from the journal site: <https://doi.org/10.1016/j.vaa.2017.08.004>

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The full details of the published version of the article are as follows:

TITLE: Sedative effects of intramuscular alfaxalone in pet guinea pigs (*Cavia porcellus*)

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JOURNAL: *Veterinary Anaesthesia and Analgesia*

PUBLISHER: Elsevier

PUBLICATION DATE: 18 September 2017 (online)

DOI: 10.1016/j.vaa.2017.08.004

1 **Sedative effects of intramuscular alfaxalone in pet guinea pigs (*Cavia porcellus*)**

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19 of manuscript. C.A.: Design, data interpretation, statistical analysis and preparation of

20 manuscript.

21

22 **Running title:** Alfaxalone in guinea pigs

23

24 **Acknowledgements:** The authors gratefully acknowledge the staff of Clinica Veterinaria

25 "VETLAN" and Clinica Veterinaria Animalia for their support to this work.

1 **Abstract**

2 **Objective** To evaluate the efficacy and side effects of alfaxalone administered
3 intramuscularly (IM) as a sedative agent in guinea pigs undergoing survey radiographs.

4 **Study design** Prospective clinical trial.

5 **Animals** Thirty client-owned guinea pigs

6 **Methods** Following baseline assessments, 5 mg kg⁻¹ alfaxalone was administered IM. Heart
7 rate, arterial haemoglobin oxygen saturation, respiratory rate, rectal body temperature,
8 palpebral reflex, response to toe and ear-pinch, righting reflex, posture, jaw tone, and reaction
9 to manipulation were assessed before and after sedation, at 5-minute intervals. The time
10 elapsed from onset of sedation to return of locomotion and coordinated limbs movements, the
11 quality of recovery and the occurrence of undesired effects were observed and recorded.

12 **Results** The mean \pm standard deviation onset of sedation was 2.7 \pm 0.6 minutes. The
13 physiological variables stayed within normal ranges until completion of the procedure.
14 Palpebral reflex and responsiveness to both ear and toe pinch were maintained during
15 sedation. Neither hypoxaemia nor hypothermia were observed. The duration of sedation was
16 29.3 \pm 3.2 minutes. Sedation and recovery were uneventful and adverse effects were not
17 observed.

18 **Conclusion and clinical relevance** In conclusion, 5 mg kg⁻¹ of IM alfaxalone represents a
19 valuable sedation protocol for healthy guinea pigs undergoing minor non-invasive
20 procedures. Further trials are required to investigate its cardiovascular effects, its clinical
21 usefulness in unhealthy patients and its combined use with analgesics for procedures
22 associated with nociception.

23 **Keywords** Sedation, alfaxalone, Anaesthesia, guinea pigs

24

25

26 Introduction

27 Radiographic examination is an important diagnostic method to identify dental,
28 gastrointestinal, respiratory, and urogenital conditions that are common in guinea pigs (*Cavia*
29 *porcellus*) (Zwingerberger & Silverman 2009; Fischetti 2012). However, in order to obtain
30 high-quality diagnostic images, sedation is frequently required to achieve immobility. Several
31 drug combinations have been used to anaesthetize or sedate laboratory guinea pigs (Dang et
32 al. 2008; Schmitz et al. 2016).

33 Nevertheless, there is a paucity of literature regarding the anaesthetic management of these
34 small rodents in a clinical context. Short-term inhalational anaesthesia with either isoflurane
35 or sevoflurane, as well as injectable anaesthesia achieved with α -2 agonists and ketamine,
36 alone or in combination, have both been described to obtain diagnostic imaging in rodents
37 and small mammals (Zwingerberger & Silverman 2009; Fischetti 2012; Hawkins & Pascoe
38 2012). However, none of them can be considered to be optimal for guinea pigs in terms of
39 effectiveness, reliability, safety and reversibility. Inhalational agents may cause dose-
40 dependent hypotension, airway irritation and, in guinea pigs, even sudden death during
41 procedures that may result from increased adrenergic tone (Flecknell 2009; Overholser et al.
42 2010). Benzodiazepines result in effective sedation and immobility, but have no analgesic
43 properties and animals can be easily aroused by nociceptive stimulation or noises (Flecknell
44 2009). Alpha-2-agonists have unpredictable effects in guinea pigs, when administered alone
45 or in combination with ketamine (Richardson & Flecknell 2009). Finally, ketamine alone
46 does result in immobilization in guinea pigs, but has been reported to cause cutaneous
47 irritation and even muscle necrosis when administered subcutaneously or intramuscularly
48 (IM) (Flecknell 2009; Richardson & Flecknell 2009).

49 Alfaxalone is a neuroactive steroid derivative of pregnanedione acting on the gamma-
50 aminobutyric acid (GABA) receptors. Its effects on ileal GABA-induced contractions have

51 been previously investigated in guinea pigs (Ong et al. 1988). The new alfaxalone
52 formulation with hydroxypropyl β cyclodextrin, licensed in many countries for intravenous
53 (IV) use as an anaesthetic induction agent in dogs and cats, has been successfully used, alone
54 or in combination with other drugs, in exotic captive species (Jones 2012). These include
55 amphibians (McMillan & Leece 2011; Posner et al. 2013; Sladakovic & Robert 2014; Adami
56 et al. 2015; Adami et al. 2016 a, b), reptiles (Bertelsen & Sauer 2011; Knotek 2014), and
57 mammals (Marsh et al. 2009; Huyhn et al. 2015; d'Ovidio et al. 2015). Alfaxalone can also
58 be administered IM to minimize stress associated with handling, physical examination, minor
59 procedures (such as IV cannulation) and in fractious animals (Marsh et al. 2009; Huyhn et al.
60 2015; Buisman et al. 2016; Khenissi et al. 2016). At a dose of 4-6 mg kg⁻¹, IM alfaxalone
61 produced a rapid and smooth anaesthetic induction in rabbits, followed by excellent recovery
62 (Huyhn et al. 2015).

63 The purpose of this study was to evaluate the efficacy and safety of IM alfaxalone as a
64 sedative agent in client-owned guinea pigs undergoing survey radiographs, for screening of
65 subclinical dental and respiratory diseases. Our hypothesis was that IM alfaxalone would
66 produce safe and adequately deep sedation in guinea pigs, suitable for short diagnostic
67 procedures requiring immobility.

68

69 **Materials and Methods**

70 **Animals**

71 Thirty client-owned guinea pigs, belonging to the same breeder and scheduled for survey
72 radiographs for screening of subclinical dental and respiratory diseases, were enrolled in this
73 prospective clinical trial. Health status was assessed before sedation with physical
74 examination (to assess general health conditions) and faecal examination (to detect
75 subclinical intestinal parasitic infections). The study was conducted under approval of the

76 Clinical Research Ethical Review Board of the Royal Veterinary College (license number:
77 URN 2016 1560), and signed informed owner consent.

78 **Procedures**

79 Animals were admitted 24 hours before commencing the diagnostic procedures to allow for
80 acclimatization, and housed in groups of five littermates of the same sex in 190x150x200cm
81 cages. They were provided with pelleted guinea pig feed, ad libitum hay and water, and fresh
82 vegetables daily. All guinea pigs were attributed an American Society of Anaesthesiologists
83 (ASA) score based on physical and copromicroscopic exams. Animals were not fasted
84 before the procedure. Baseline values for heart rate (HR, from chest auscultation) pulse rate
85 (pulse oximeter transducer), arterial haemoglobin oxygen saturation (SpO₂, from pulse
86 oximeter transducer placed digits of pelvic limb), respiratory rate (f_R) from observation of
87 thoracic excursion) and rectal temperature (T) were obtained in each guinea pig before
88 sedation (T₀). As a part of the baseline assessment, the following variables were also assessed
89 in the awake animals: palpebral reflex, response to toe and ear-pinch, righting reflex, posture,
90 jaw tone, and reaction to manipulation. The palpebral reflex was assessed with a gentle tactile
91 stimulation of the upper eyelid; the possible outcomes were yes (present) or no (absent). The
92 response to toe- and ear-pinch was evaluated by applying blunt surgical forceps, for a
93 maximum of two seconds, at the level of the distal interphalangeal junction and of the base of
94 the ear, respectively, with a score ranging from 0 to 2 where (0) indicated limb
95 withdrawal/head movement immediately after pinching (intense response), (1) delayed limb
96 withdrawal/head movement (more than 1 second after stimulus application), and (2) no
97 response. The righting reflex was assessed after the guinea pigs had been placed by the
98 observer in dorsal recumbency over a flat, firm surface. A 0-3 score was used, where (0)
99 indicated that the animal regained sternal recumbency immediately after positioning, (1) it
100 regained sternal recumbency within 5-10 seconds after positioning, (2) it attempted to regain

101 sternal recumbency but failed to achieve sternal position, and (3) it maintained dorsal
102 recumbency with no attempts to reposition. Posture was evaluated with a score ranging from
103 0 to 5, as follows: (0) normal, (1) head up but sitting, (2) head down and sternal recumbency,
104 (3) lateral recumbency, (4) dorsal recumbency but responsive to stimulation, and (5) dorsal
105 recumbency and no response to stimulation. Jaw tone was evaluated with a 0-2 score, where
106 0, 1 and 2 were indicative of absent, decreased, and normal jaw tone, respectively. Finally,
107 reaction to manipulation was assessed with a score ranging from 0 to 2, where 0 was
108 indicative of normal reaction, 1 of decreased response, and 2 of absent response.

109 Following baseline assessments, 5 mg kg⁻¹ (0.5 mL kg⁻¹) alfaxalone (Alfaxan 1%; Jurox, UK)
110 was administered IM in the left or right quadriceps femoris. The animals were manually
111 restrained during intramuscular injection. Time to onset of sedation, defined as the minutes
112 elapsed from IM injection to lateral recumbency, was recorded. At this point, the diagnostic
113 procedure was commenced. An electric heating pad (Eickwarm; Eickemeyer, Italy) was used
114 to prevent hypothermia. During sedation, the same variables assessed during baseline
115 evaluation were recorded at the following 7 time points: T5, T10, T15, T20, T25, T30 and
116 T35, indicative of 5, 10, 15, 20, 25, 30 and 35 minutes after injection, respectively. Time to
117 recovery, defined as the minutes elapsed from onset of sedation to return of locomotion and
118 coordinated limb movements was recorded, as well as the duration of the clinical procedure
119 (minutes). The time elapsed from onset of sedation to time of recovery was defined as
120 duration of sedation. The occurrence of adverse effects, namely hypoxaemia (defined as
121 SpO₂ < 97%), severe cardiorespiratory depression (defined as decrease in the basal values for
122 HR and f_R by 50% or more), hypothermia ($T < 37.2$ °C), delayed food intake (more than one
123 hour after recovery), and gastro-intestinal disturbances observed within 72 hours of sedation,
124 was recorded. After the end of the diagnostic procedure, the guinea pigs were allowed to
125 recover in individual boxes in a quiet room. Fresh vegetables and drinking water were offered

126 at recovery and the guinea pigs were discharged from the hospital after the observation
127 period.

128

129 **Statistical analyses**

130 Normality was assessed with the Kolmogorov-Smirnov test, and with the Shapiro-Wilk test.

131 Data were then analysed with either one-way repeated measures analysis of variance,

132 followed by Holm-Sidak method for multiple comparisons versus baseline (T0), or with

133 Friedman repeated measures analysis of variance on ranks, followed by Tukey test for

134 multiple comparisons, where it applied. The time point was used as group factor.

135 Commercially available software was used for statistics (SigmaStat and SigmaPlot; Systat

136 Software Inc., CA, USA). *P* values < 0.05 were considered statistically significant. Data are

137 presented as either mean \pm standard deviation or median (range), where it applies.

138

139 **Results**

140 A total of 15 male and 15 females guinea pigs that weighed 456 (320-930) grams and were 8

141 (3-12) months old were enrolled in the study and judged healthy based on physical and

142 copromicroscopic exams. All guinea pigs were assigned an ASA classification risk I. Data

143 for HR and onset and duration of sedation and duration of the clinical procedure were

144 normally distributed. All the guinea pigs showed a reaction to IM injection of alfaxalone,

145 characterized by twitches of the lumbar muscles and attempts to fight physical restraint. The

146 onset of sedation was 2.7 ± 0.6 minutes. Heart rate significantly increased after IM alfaxalone

147 compared to baseline (from 226 ± 26 to 235 ± 30 beats minute^{-1}), and there was a statistically

148 significant difference between the values recorded at T0 (baseline) and all the other time

149 points except T15 ($p < 0.001$). There were no statistically significant differences in T (which

150 ranged between $37.2\text{-}39.5^\circ\text{C}$) and *f*R [which ranged from 85 (75-98) to 89 (78-100) breaths

151 minute⁻¹] between baseline and the other time points (Figs. 1–3). All the physiological
152 variables stayed within normal ranges for the species (Flecknell 2002; Keeble 2009) until
153 completion of the procedure. Similarly, SpO₂ remained above 96% throughout the procedure
154 and hypoxaemia was never observed. The scores for righting reflex, reaction to manipulation
155 and jaw tone increased, compared to baseline, during the first 15 minutes of sedation, and
156 decreased again after T15. With respect to the aforementioned variables, there were
157 statistically significant differences between T0 and T5, T10 and T15 ($p < 0.001$; Table 1). The
158 scores for posture increased and remained higher than baseline values until T20 ($p < 0.001$).
159 Palpebral reflex and responsiveness to both ear and toe pinch were maintained during
160 sedation in all animals. In all cases, the depth and the duration of sedation (29.3 ± 3.2
161 minutes) achieved with IM alfaxalone were sufficient to allow completion of the clinical
162 procedure, which lasted 30.0 ± 4.5 minutes. The survey radiographs did not reveal
163 abnormalities in any of the guinea pigs enrolled in the trial. Sedation and recovery were
164 uneventful and adverse effects were not observed. In addition, all animals showed normal
165 appetite and regular defecation within one hour following the procedure.

166

167 **Discussion**

168 This study has confirmed our hypothesis that 5 mg kg⁻¹ alfaxalone IM represents a valuable
169 alternative to previously reported sedation protocols for guinea pigs undergoing minor
170 clinical procedures (Zwingerberger & Silverman 2009; Fischetti 2012; Hawkins & Pascoe
171 2012). Indeed, the effects of IM alfaxalone are species-dependent. Whilst in various
172 mammalian species it is reported to produce reliable sedation when administered either alone
173 or in combination with other drugs (Huyhn et al. 2015; d'Ovidio et al. 2015), in chelonians it
174 appears to be less effective (Scheelings 2013). The depth of sedation achieved in the guinea
175 pigs enrolled in the current study was sufficient to ensure immobility, ease to positioning and

176 adequate muscle relaxation for at least 15-20 minutes, which is the average time required for
177 most diagnostic studies at our institution. The minimal standard deviation also shows
178 consistency with respect to the rapid onset of action and the duration of the sedative effect,
179 which may indicate predictability of this sedation protocol in guinea pigs. Moreover,
180 respiratory function and body temperature were preserved throughout the procedure, which
181 suggests that IM alfaxalone may be a clinically useful anaesthetic choice in healthy guinea
182 pigs.

183 Overall, alfaxalone provided good quality of sedation compared to other anaesthetic protocols
184 previously described in guinea pigs. A study comparing ketamine–xylazine (administered
185 subcutaneously, IM, or intraperitoneally), intraperitoneal pentobarbital, and IM
186 medetomidine, found that reliable immobilization and absence of response to blood sampling
187 were not achieved. Moreover, time to recovery ranged from 49.0 to 294.3 minutes (Dang et
188 al. 2008). Recovery from alfaxalone sedation was smooth and uneventful, and none of the
189 animals showed hypothermia in contrast with previous reports (Schmitz et al. 2016).

190 It was challenging during the current study was to distinguish between deep sedation and
191 general anaesthesia. Alfaxalone is an induction agent capable of producing both general
192 anaesthesia and deep sedation, depending on the dose and the route of administration.
193 However, sedation is commonly regarded as the preferred option for non-invasive clinical
194 procedures of short duration in small animals, and very often owners raise some concerns
195 when general anaesthesia is proposed instead. In order to define the anaesthetic effects of IM
196 alfaxalone, it was decided to evaluate both righting reflex and posture. In laboratory rodents,
197 the loss of righting reflex is unarguably considered the cut off parameter between sedation
198 and anaesthesia, as it is believed to imply unconsciousness (Flecknell 2009). Whilst certain
199 diagnostic procedures do not necessarily require unconsciousness, other features are desirable
200 for the purpose of obtaining good quality images, especially when procedures carrying a

201 potential risk for personnel safety (e.g. radiographic examinations) are to be performed.
202 Among these features, adequate sedation and muscle relaxation are desired to achieve ease to
203 positioning and immobility. The guinea pigs remained in lateral recumbency for a period up
204 to 20 minutes in the absence of stimulation, but most of them tended to regain their righting
205 reflex within 15 minutes after injection. This seems to indicate that unconsciousness - and
206 therefore general anaesthesia - may be achieved in guinea pigs after IM alfaxalone at the dose
207 investigated in this study, but the anaesthetic effects wear off progressively, transitioning to a
208 state of deep sedation. Increasing the alfaxalone dose may deepen or prolong its anaesthetic
209 effects in guinea pigs. However, investigating the effect of a higher doses was beyond the
210 aims of this study.

211 Increasing the dose of IM alfaxalone would also increase the risk for cardiorespiratory side
212 effects, as has been demonstrated in dogs, cats and rabbits (Huynh et al. 2015; Tamura et al.
213 2015a; Tamura et al. 2015b). Moreover, because alfaxalone is only available in Europe at a
214 concentration of 10 mg mL⁻¹ doses higher than 5 mg kg⁻¹ would result in unacceptably high
215 IM injection volumes for small rodents. It has been demonstrated that histopathological
216 lesions of the skeletal muscles may result not only from the drug's chemical characteristics,
217 but also from mechanical compression when large volumes are injected (Evans 2005;
218 Thuillez et al. 2009). This represents a major limitation when dealing with smaller mammals
219 and, as previously advocated by other investigators, the commercialization of more
220 concentrated solutions might partially overcome this issue (Tamura et al. 2015 b).

221 All the guinea pigs showed a behavioural reaction to IM injection of alfaxalone. However,
222 whether this reaction was caused by the drug itself or by the needle insertion cannot be
223 determined. No adverse reactions (e.g. self mutilation) were noticed in the present study in
224 the days following the procedure. However, one drawback of alfaxalone compared to other
225 injectable agents is the lack of reversibility of the effects, as no antagonists were available at

226 the time of writing.

227 All the guinea pigs maintained unaltered response to both ear and toe pinch throughout the
228 procedure, which indicates that analgesia was not achieved. This limits the clinical of
229 alfaxalone as a sole agent to minor, non-invasive clinical procedures.

230 Although to the best of the authors' knowledge no pulse oximetry device has been validated
231 for guinea pigs, the fact that SpO₂ remained above 96% may indicate that severe hypoxaemia
232 did not occur despite the lack of oxygen supplementation. As rodents are predisposed to
233 respiratory diseases, the use of anaesthetic agents with minimal impact on the respiratory
234 function is essential.

235 Despite increased HR after administration of alfaxalone, the measured cardiorespiratory
236 variables were within normal reference ranges for the species (Flecknell 2002; Keeble 2009),
237 with no remarkable changes, in the majority of the animals enrolled. With respect to the
238 cardiovascular function, it is worth to consider that a more comprehensive evaluation of the
239 latter would imply at least the monitoring of the arterial blood pressure. Previous studies
240 conducted in cats and dogs have shown that doses of alfaxalone higher than 5 mg kg⁻¹ caused
241 cardiovascular depression characterised by decreased mean arterial pressure in the absence of
242 changes in the heart rate (Tamura et al. 2015a; Tamura et al. 2015b). Unfortunately,
243 monitoring of systemic arterial blood pressure was not performed in this study.

244 The guinea pigs recruited for this study were aged 8 (3-12) months and were purchased from
245 the same breeder. Considering that guinea pigs become sexually mature at the age of 3
246 months, the study population was mainly composed of adults, with a few younger animals.
247 This may represent an unintentional selection bias, as in the present study the protocol was
248 not tested against the neonates or geriatric animals, in which alfaxalone may have sensibly
249 different pharmacokinetics and pharmacodynamics. Moreover, the same origin of the animals
250 may imply a similar genetic background, an aspect which may limit the validity of our

251 findings to a sample poorly representative of guinea pigs in general.

252 In conclusion, IM alfaxalone may represent a useful means to provide deep sedation
253 with minimal side effects to healthy guinea pigs undergoing diagnostics and minor non-
254 invasive procedures. Further trials are required to investigate its cardiovascular effects, its
255 clinical usefulness in unhealthy patients and its combined use with analgesics for invasive
256 procedures. Finally, studies recruiting animals coming from different populations should be
257 encouraged to investigate the inter-individual variability.

258

259

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Figure 2 Means and standard deviations of respiratory rates, measured by direct visualization of the thoracic excursion before and after intramuscular injection of alfaxalone, in 30 guinea pigs undergoing survey radiographic examination. T5, T10, T15, T20, T25, T30 and T35 are minutes after injection; T0 is the baseline (before injection).

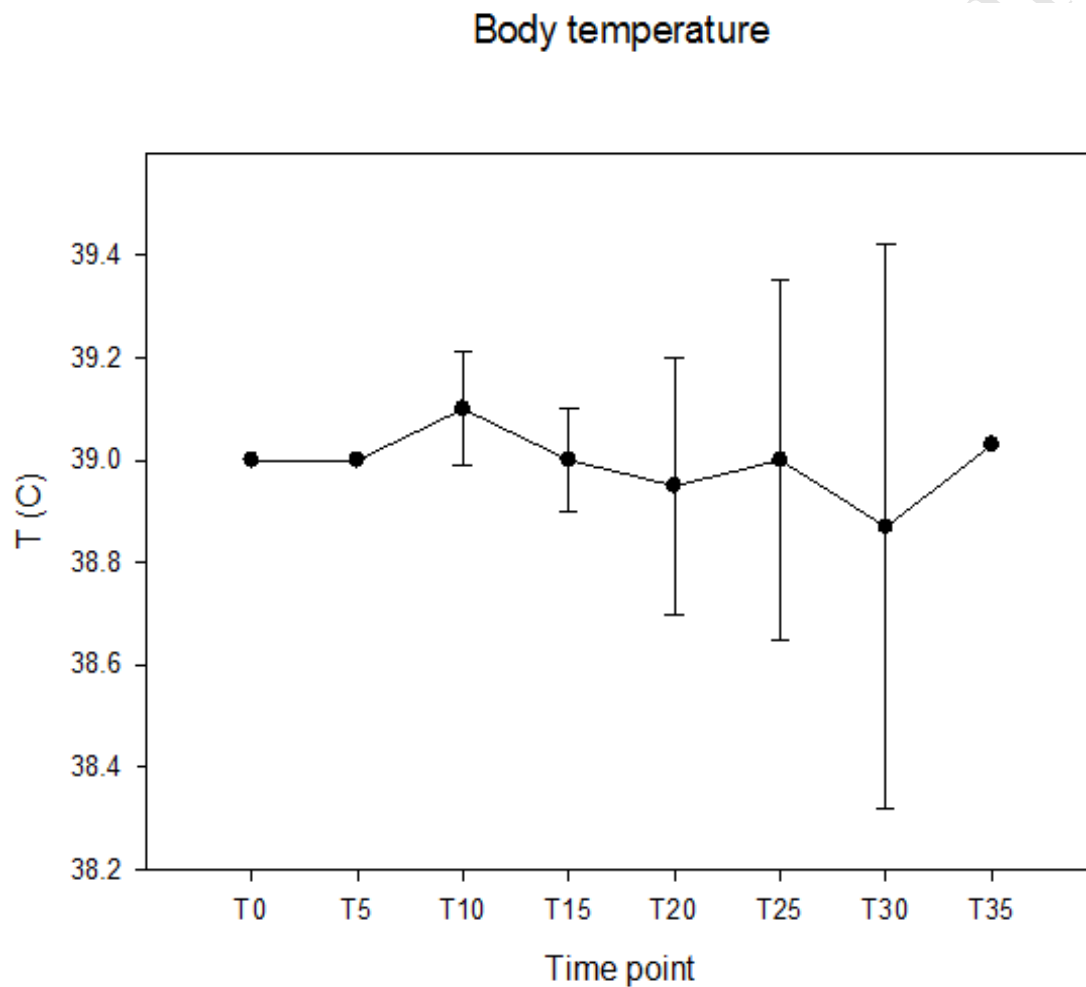
Figure 3 Means and standard deviations of heart rates, measured by thoracic auscultation before and after intramuscular injection of alfaxalone, in 30 guinea pigs undergoing survey radiographic examination. T5, T10, T15, T20, T25, T30 and T35 are minutes after injection; T0 is the baseline (before injection).

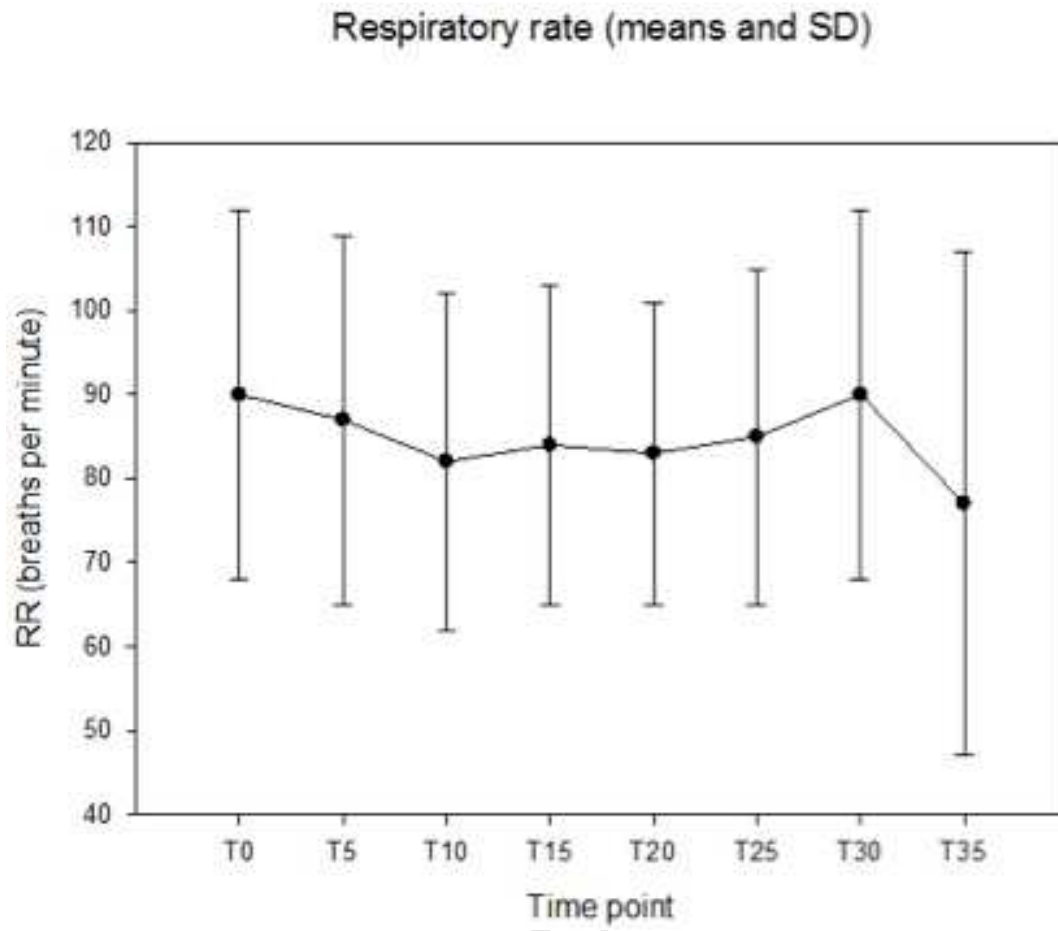
Table 1 Variables assessed during sedation with 5 mg kg⁻¹ intramuscular alfaxalone in thirty guinea pigs undergoing survey radiographic exam. The time points T5, T10, T15, T20, T25, T30 and T35 are minutes after injection and *n* is the number of animals in which the clinical procedure was completed at the respective time point. Data are reported as median (range).

Timepoint	Righting reflex (0-3)	Reaction to manipulation (0-2)	Jaw tone (0-2)	Posture (0-5)	Palpebral reflex (yes or no)	Response to ear pinch (0-2)	Response to toe pinch (0-2)
T0 (<i>n</i> =30)	0 (0-1)	0 (0-1)	2 (1-2)	0 (0-4)	yes	0 (0-0)	0 (0-0)
T5 (<i>n</i> =30)	2 (1-2) *	2 (1-2) *	1 (1-2) *	4 (3-5) *	yes	0 (0-0)	0 (0-0)
T10 (<i>n</i> =30)	2 (1-2) *	2 (1-2) *	1 (1-1) *	4 (4-5) *	yes	0 (0-0)	0 (0-0)
T15 (<i>n</i> =30)	2 (1-2) *	2 (1-2) *	1 (0-1) *	4 (3-5) *	yes	0 (0-0)	0 (0-0)
T20 (<i>n</i> =30)	1 (1-1)	1 (1-1)	1 (1-2)	3 (3-4)	yes	0 (0-0)	0 (0-0)
T25 (<i>n</i> =27)	0 (0-1)	0 (0-1)	2 (1-2)	2 (1-3)	yes	0 (0-0)	0 (0-0)
T30 (<i>n</i> =18)	0 (0-1)	0 (0-1)	2 (1-2)	0.5 (0-1)	yes	0 (0-0)	0 (0-0)
T35 (<i>n</i> =6)	0 (0-1)	0 (0-1)	2 (1-2)	0.5 (0-1)	yes	0 (0-0)	0 (0-0)

*Significantly different from baseline (T0). Righting reflex ranged from (0) the animal regained sternal recumbency immediately after positioning, to (3) it maintained dorsal recumbency with no attempts to reposition. Reaction to manipulation ranged from (0) normal reaction to (2) absent response. Jaw tone ranged from (0) absent to (2) normal. Posture ranged from (0) normal to (5) dorsal recumbency and no response to stimulation. Response to ear pinch and toe pinch ranged from (0) intense response to (2) no response.

Figure 1: Means and standard deviations of rectal body temperature, measured before and after intramuscular injection of alfaxalone, in 30 Guinea pigs undergoing survey radiographic examination. T5, T10, T15, T20, T25, T30 and T35 are minutes after injection; T0 is the baseline (before injection).





ACCEPTED

Heart rate (means and SD)

