Paper

Clinical parameters and adrenocortical activity to assess stress responses of alpacas using different methods of restraint either alone or with shearing

T. Wittek, T. Salaberger, R. Palme, S. Becker, F. Hajek, B. Lambacher, S. Waiblinger

Shearing of alpacas is stressful and is undertaken by restraint in the standing position, cast on the floor or on a tilt table. The objectives of the study were to evaluate and compare the stress responses between different methods. The study consisted of two parts. In part one, 15 animals were restrained applying all three methods but without shearing. In part two, 45 animals in three groups of 15 were shorn using one of the three procedures. Body temperature, heart rate, respiratory rate, salivary cortisol and faecal cortisol metabolites (FCM) were measured. Part 1: restraint in a standing position was less stressful than other procedures. Part 2: the classic clinical parameters changed significantly over time but without significant differences between the methods. The number of injuries did not differ. Saliva cortisol and FCM concentrations varied in wide ranges between animals. An increase in FCM concentrations occurred in all groups but saliva cortisol concentration increased only after shearing on the ground. The recommendations of the study are to shear calm alpacas in the standing position but animals showing severe defence reactions should be shorn either cast on the ground or on a table to decrease the risk of injuries.

Introduction

Shearing of alpacas is associated with a major change in their daily routine involving the gathering and handling of the animals even prior to restraint. McEwen (2000) defined stress as a real or interpreted threat to the physiological or psychological integrity of an individual that results in physiological and/or behavioural responses. In sheep, it has been described in a number of studies (Hargreaves and Hutson 1990a, Mears and others 1999, Carcangiu and others 2008, Lexen and others 2008, Piccione and others 2008, 2011, Sanger and others 2011). Together they have described changes of various clinical, haematological and biochemical parameters such as body temperature, heart rate, haematocrit, glucose, cortisol, growth hormone and endorphin concentration. However, little is available in the literature on stress response associated with shearing in camelids. Bonacic and Macdonald (2003) investigated the impact of captivity, handling and shearing in vicunas and found similar changes to those in 'stressed' sheep. Carmanchahi and others (2011) published a report on physiological response in guanacos which had been captured and shorn. The studies showed that stress increased with increasing handling time. However, the results of the two studies which were performed in vicunas and guanaco which are non-domesticated animals might not be transferable to domesticated camelids like alpacas which are used to be handled by people.

Different methods of restraint and shearing are applied by alpaca farmers, which may result in different amount of stress responses and may have different impact on animal behaviour and welfare (Wiede 2014). These issues have been a matter of heated discussion among alpaca farmers, veterinarians and animal welfare organisations despite no reliable data being available to support the opinions of the stakeholders.

To assess the impact of the manipulations during shearing on the animals, various clinical, laboratory and behavioural parameters can be evaluated. In particular, saliva cortisol is considered to reflect a short-term stress response, whereas faecal cortisol metabolites (FCM) are an estimate for longer lasting stress responses (Möstl and Palme 2002). These measurement techniques have been used in numerous animal species, but have also been validated and used in new world camelids (Anderson and others 1999, Sheriff and others 2011 Arias and others 2013).

The aims of the study were to assess and compare different methods of restraint and shearing of alpacas according to the stress response of the animals and to develop recommendation from the results, if possible. It was hypothesised that restraint and shearing of the animals generally cause stress reactions but

Veterinary Record (2017) doi: 10.1136/vr.104232

T. Wittek, Prof Dr Med Vet Habil, DiplECBHM,
S. Becker, Cand Med Vet,
B. Lambacher, Dr Med Vet,
University Clinic for Ruminants, University of Veterinary Medicine Vienna, Vienna, Austria
T. Salaberger, Dr Med Vet,
R. Palme, Prof Dr Med Vet Habil,
Unit of Physiology, Pathophysiology and Experimental Endocrinology, University of Veterinary Medicine Vienna, Vienna, Austria
F. Hajek, Mag Med Vet,
S. Waiblinger, Prof Dr Med Vet Habil,
Institute of Animal Husbandry and Animal Welfare, University of Veterinary Medicine Vienna, Vienna, Austria

E-mail for correspondence: Thomas.Wittek@vetmeduni.ac.at

Provenance: Not commissioned; externally peer reviewed

Accepted February 16, 2017

that the severity differs between methods. We investigated both restraint alone and in combination with shearing.

Materials and methods

The study consisted of two parts to allow separate assessment of restraint alone (without shearing) in contrast to restraint and shearing. Animals were restrained but not shorn in part 1 of the study; in part 2 animals were restrained and shorn. All manipulations and shearing were performed by the owners of the animals or experienced farm workers. The restraint of the animals was performed in three different ways:

1. In standing position restrained by two assisting persons (Fig 1).
2. On a mattress on the ground using ropes on front and hind limbs; the head was held on the floor by one assisting person (Fig 2).
3. On a tilt table which is specially designed for alpaca shearing using ropes on front and hind limbs; the head was held on the table by one assisting person (Fig 3).

The study was approved by the institutional ethics and animal welfare committee (Vetmeduni Vienna) in accordance with Good Scientific Practice (GSP) guidelines and national legislation (part 1 Austria: BMWF GZ 68.205/0081-WF/V/3b/2015, part 2 Germany: Federal State Brandenburg, GZ 2347-11-2015).

Animals

Part 1 was performed on farm with approximately 120 animals in Austria; part 2 on a farm with approximately 250 animals in Germany. In both farms, the animals were familiar with the persons on the farms and used to be handled.

The study was performed in male (part 1) and female (part 2) alpacas. All animals except two yearlings in part 1 (12 and 14 months) had been shorn at least once before. The alpacas were of identical sex in each part of the study since it has been reported that sex of the animals affects the stress response after handling and shearing in guanacos. In contrast, age did not appear to influence stress response (Carmanchahi and others 2011). The female alpacas in part 2 had given birth to crias between three weeks and two months before shearing. Mating season had already started without the animals being tested for pregnancy by that time.

The sample size has been derived from similar studies in sheep in which 10–20 animals were included in each treatment group (Carcangiu and others 2008, Piccione and others 2008, 2011). The decision which animals of the herds were included in the study was made by drawing lots. A clinical examination had been performed before the animals entered the study to exclude animals which were not clinically healthy. Exclusion parameters were decreased feed intake, decreased general behaviour, increased body temperature, heart and respiratory rate above the upper physiological range or injuries. Both parts of the study were performed during May and June 2015.

Part 1

The three restraint methods (without shearing) were applied on 15 alpacas. Every animal was restrained using all three methods (crossover study) in a random order applying a Latin square design (3×5). The animals were restrained for 15 minutes, which was the estimated time period necessary for shearing. The time between the applications of the three methods was one week.

Part 2

Each restraint method was also applied in 15 animals. Since the animals were restrained and shorn, 45 animals in total had to be used in part 2. The 45 alpacas were allocated to the three methods by drawing lots. Fifteen animals were investigated per day on three consecutive days. Each day all three restraint methods were used following a Latin square design (3×5). Duration of shearing and injuries caused by shearing were recorded.

Physical examination

Heart rate was measured by auscultation, respiratory rate by counting excursion of the rib cage and body temperature by measuring rectal temperature using a digital thermometer (Kruuse DIGI-VET SC 12, Langeskov, Denmark) before, during and after the manipulations. Time points were defined t0, t5, t10, t20, t30, t40 and t60 (0, 5, 10, 20, 30, 40 and 60 minutes after start of manipulation).

Salivary cortisol and FCMs

Saliva was obtained from all animals before any other manipulation (t0) and at 20, 40 and 60 minutes (t20, t40, t60) after restraint (and shearing in part 2) using a commercial saliva

FIG 1: Shearing of an alpaca in standing position restrained by two assisting operators

FIG 2: Shearing of an alpaca restrained on a mattress on the floor fixated using ropes on front and hind limbs

FIG 3: Shearing on the tilt table specially designed for alpaca shearing, the alpaca is restrained to the table using ropes on front and hind limbs; the table is then moved into a horizontal position

TABLE 1: Clinical parameters (arithmetic mean±sd) over time (0, 5, 10, 20, 30 40 and 60 minutes) in alpacas which had been restrained using three different methods (part 1)

Parameter	Restraint	t0	t5	t10	t20	t30	t40	t60
Heart rate/minute	Standing	80±12	72±12	72±15	72±10	80±16	76±18	76±23
	On the ground	72±14	68±15	68±13	76±19	76±22	80±14	68±13
	On the table	92±18	72*±13	72*±11	76±14	80±14	80±17	76±16
Respiratory rate/minute	Standing	29±8	28±10	28±12	30±14	28±8	22±16	24±7
	On the ground	32±9	36±8	34±8	38*±8	26±13	24±10	24±14
	On the table	30±15	36±9	32±8	34±10	30±11	27±9	26±10
Body temperature (°C)	Standing	37.9±0.6	37.9±0.6	38.2±0.6	38.0±0.5	38.1±0.5	38.1±0.4	38.0±0.4
	On the ground	38.1±0.5	38.2±0.6	38.1±0.7	38.2±0.8	38.2±0.7	38.3±0.6	37.9±0.6
	On the table	37.6±0.6	37.8±0.6	37.6±0.6	37.9±0.6	37.9±0.7	37.9±0.5	38.2±0.3

*Indicates significant difference from t0, no differences between restraint methods

sampling kit (Salivette, Sarstedt, Germany). The sampling points had been derived from the study of Anderson and others (1999), who found that at the commonly used sampling time of 30 minutes, the peak of cortisol concentration has not been reached in all animals. Samples were frozen (–21°C) immediately and stored until analysed. Faeces were obtained from the rectum at the start of the manipulation with the animals and 33 hours later. This time lag in FCM excretion has been reported in new world camelids (Arias and others 2013). Analyses of saliva and faeces were performed using a cortisol and 11-oxo-etiocholanolone enzyme immunoassays as described in detail by Arias and others (2013). All laboratory analyses have been performed within 4 months after sampling.

Statistical analysis

Data are provided as arithmetic mean and sd (AM±sd). Normal distribution has been tested using the Kolmogorov-Smirnov test. Since some parameters were not normally distributed, data have been log-transformed before further statistical analyses. Statistical analyses were performed separately for part 1 and part 2 of the study. Levene's test has been applied testing equality of variances. For comparison between restraint and shearing methods, clinical parameters and cortisol concentrations over time analysis of variance (repeated measures analysis) procedures have been used. Bonferroni test was applied as post hoc test if applicable. To compare FCM concentrations at t0 and t33 the Wilcoxon rank-sum statistical test was applied. P values <0.05 were considered as indicative for statistical differences. All statistical analyses were performed with the software package SPSS V.22.0.

Results

All 15 animals of part 1 could be restrained with all three methods without any problems or injuries. In part 2, one alpaca, which was supposed to be shorn in standing position, could not be restrained for shearing by two assisting persons without putting the animal and personnel in danger. The attempt was stopped after three minutes and the animal was excluded from the study. The duration of shearing was not significantly different (P=0.069) between the methods, although the means were slightly longer for those that needed cast (standing: 17.5±2.6 minutes; restrained on the floor: 19.0±2.4 minutes; restrained on the shearing table: 20.9±3.5 minutes). All injuries were superficial cuts, which only needed local disinfection and the number of these caused by shearing also did not differ significantly (P=0.418) between the groups, although in this case the means tended to an advantage for firm restraint (standing 7 of 14 animals; on the ground 3 of 15 animals; on the table 4 of 15 animals).

Clinical parameters

Part 1 (restraint without shearing)

Animals which have been restrained by a halter and rope in standing position with two assisting persons over 15 minutes showed minor, non-significant fluctuations of heart rate, respiratory rate and body temperature (Table 1). The average values of measured parameters remained within physiological ranges. A similar situation was found in animals which were cast on a mattress on the ground and in animals held down on the shearing table. With the exceptions of heart rate in animals restrained on the tilt table (t5 and t10) and the respiratory rate at t20 in animals restrained on the ground did not result in significant changes over time. There were no differences between heart rate, respiratory rate and body temperature at any time point comparing the restraint methods (Table 1).

Part 2 (restraint and shearing)

Although heart rate and respiratory rate numerically increased after beginning of restraint and shearing in all groups (Table 2), significant differences were only found in animals restrained on the ground (respiratory rate) and in animals held down on the shearing table (heart and respiratory rate). However, the parameters heart and respiratory rate remained within physiological ranges or were only slightly above them. Body temperature did not show any significant changes in all groups. No differences between measurements of heart rate, respiratory rate and body temperature were found at any time point comparing the restraint and shearing methods.

TABLE 2: Clinical parameters (arithmetic mean±sd) over time (0, 5, 10, 20, 30 40 and 60 minutes) in alpacas which restrained and shorn three different methods (part 2)

Parameter	Restraint	t0	t5	t10	t20	t30	t40	t60
Heart rate/minute	Standing	68±16	69±17	70±18	73±23	74±18	72±19	71±20
	On the ground	68±7	72±14	76±14	76±16	70±11	66±11	68±9
	On the table	68±11	70±20	68±17	76*±17	78±17	74±20	64±16
Respiratory rate/minute	Standing	35±9	46±17	45±17	41±14	38±18	38±19	32±20
	On the ground	26±11	38*±13	38±14	38±12	36±6	30±9	32±15
	On the table	26±6	36*±17	35*±18	34*±20	40±13	30±9	30±9
Body temperature (°C)	Standing	38.4±0.4	38.4±0.5	38.4±0.6	38.5±0.8	38.6±0.7	38.5±0.6	38.5±0.6
	On the ground	38.1±0.4	38.1±0.6	38.1±0.6	37.9±0.6	38.0±0.5	38.1±0.5	38.3±0.4
	On the table	38.1±0.4	38.1±0.5	38.2±0.4	38.1±0.5	38.1±0.5	38.1±0.4	38.2±0.6

*Indicates significant difference from t0, no differences between restraint methods

Salivary cortisol and FCMs

Part 1 (restraint without shearing)

Animals in all groups showed significant increases of salivary cortisol concentrations 20 and 40 minutes after start of the restraint. The cortisol concentration at 60 minutes did not differ significantly from 20 and 40 minutes, but was also different from t0 concentration (P=0.048) in animals restrained in standing position. Their saliva concentrations were not different between the groups at any sampling time (Fig 4); however, at t60 the concentration in standing animals (1.53±1.96 ng/ml) tended to be higher (P<0.1) in comparison to animals restrained on the ground (0.73±0.60 ng/ml) or on the table (0.93±1.10 ng/ml). The concentrations of FCMs were significantly increased after 33 hours. This was irrespective of the restraint method (Fig 5).

Part 2 (restraint and shearing)

The saliva cortisol concentrations tended to increase (P<0.1) in all restrained and shorn animals irrespective of method (Fig 6). However, cortisol concentrations varied very extensively between individual animals. The increase over time is significant only in the group of animals, which had been shorn restrained on the ground. Comparing the restraint methods no differences could be detected in saliva cortisol concentration. Concentrations of FCM after shearing were increased in all groups after 33 hours. As with the salivary cortisol results, FCM concentrations varied in wide ranges in all groups and did not differ between the groups (Fig 7).

Discussion

The main results of the study are that all restraint and shearing methods provoked a stress response even in animals which were used to being handled. This had been expected and described in other domestic and wild animals (Hargreaves and Hutson 1990a, Mears and others 1999, Bonacic and Macdonald 2003, Carcangiu and others 2008, Piccione and others 2008, 2011, Sanger and others 2011, Casella and other 2016). The clinical parameters and salivary cortisol concentrations in part 1 showed that the stress response was different between restraint methods, although the FCM did not. If the animals were restrained in the standing position, the results suggest that they tolerated this better than either recumbency on the mattress on the ground or on the shearing table. Two potential reasons are likely, first the forced lying position itself and secondly the restraint of hind and front limbs by ropes pulling the limbs gently but firmly caudally or cranially, respectively. These two potential factors cannot be differentiated. The simple conclusion is to avoid recumbency if possible. There were no substantial differences between the shearing methods whether on the floor or on the shearing table. Shearing on the tilt table tended to take longest; the major advantage of the table is that the animal can be shorn in a convenient height over the ground, which makes the work easier for the shearer and the assisting persons.

Although it was not intended or possible to compare between part 1 and 2 of the study, it is reasonable to believe that shearing the animals resulted in a stress response additional to that caused by restraint alone. It is reasonable to believe that the noise of the clippers and the intensive handling during shearing contribute to the additional stress response. Vocalisation in several animals during shearing but in only one during restraint alone has been observed by Waiblinger and others (2017, Effects

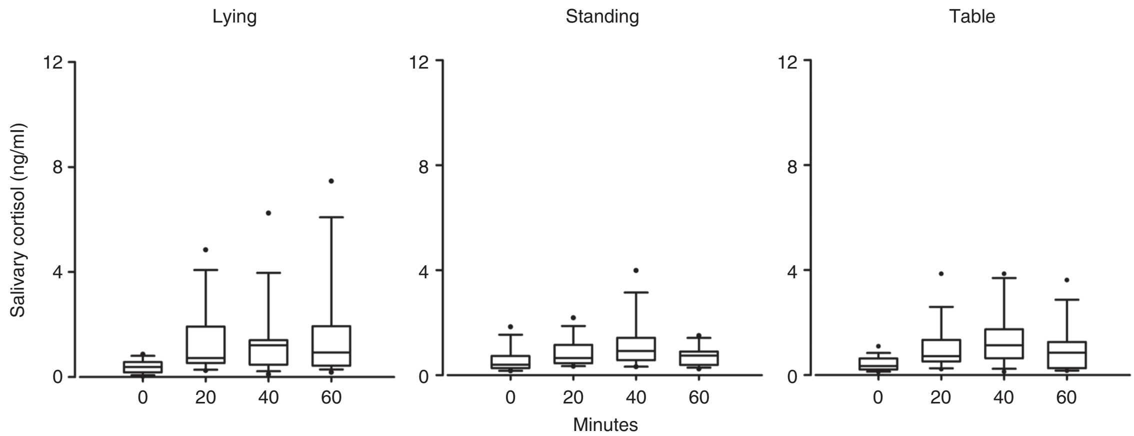

FIG 4: Salivary cortisol concentrations (ng/ml) of alpacas after different methods of restraint without shearing (part 1)

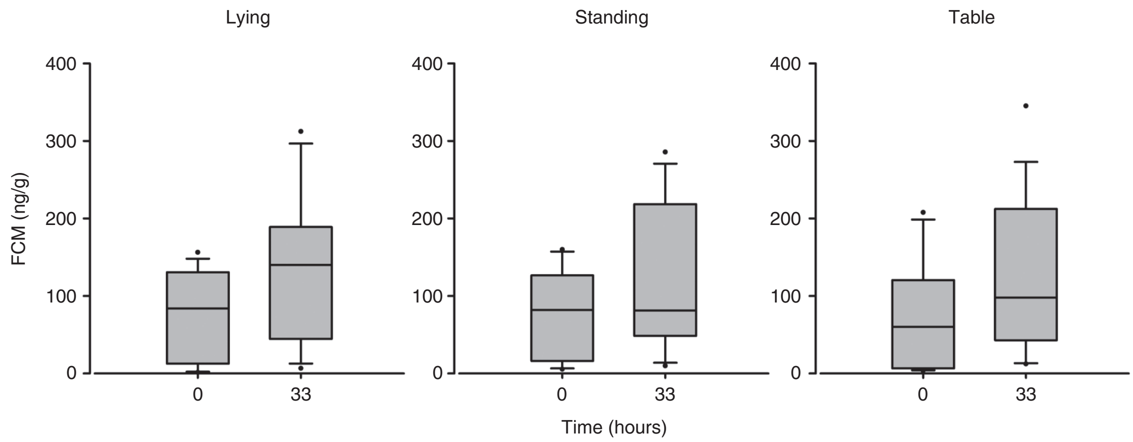

FIG 5: Concentration (ng/g) of faecal cortisol metabolites (FCM) of alpacas before and after different methods of restraint without shearing (part 1)

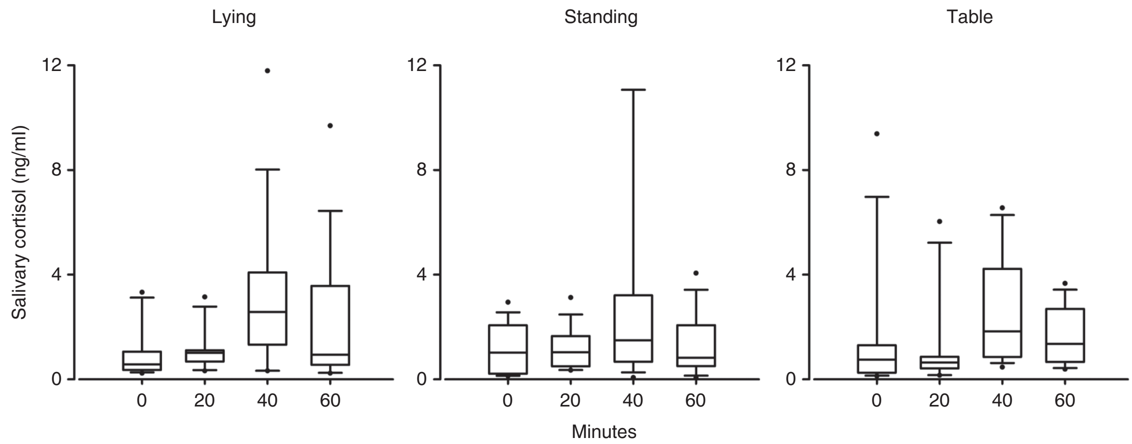

FIG 6: Salivary cortisol concentrations (ng/ml) of alpacas after different methods of restraint and shearing (part 2)

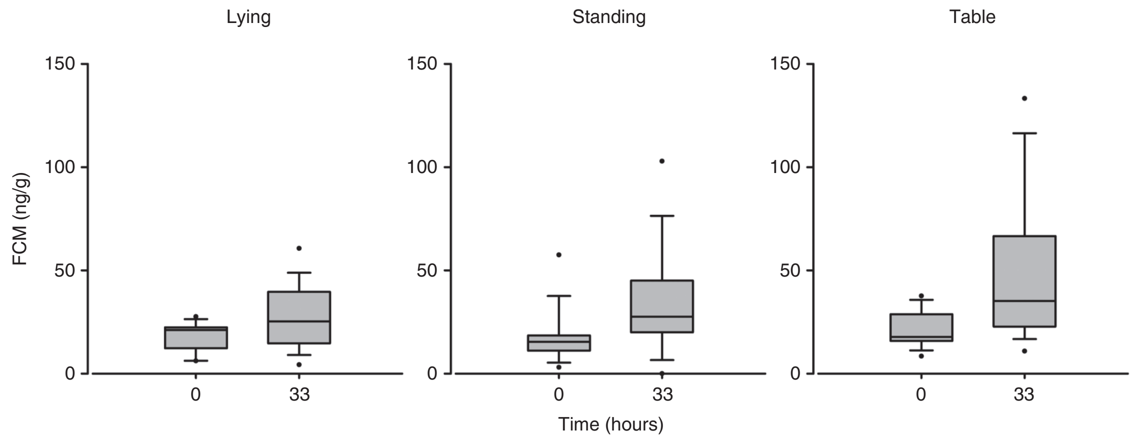

FIG 7: Concentrations (ng/g) of faecal cortisol metabolites (FCM) of alpacas before and after different methods of restraint and shearing (part 2)

of the method of restraint for shearing on behaviour, heart rate variability and eye temperature in alpacas. Applied Animal Behaviour Science (in preparation)). Additionally, the shearing took a little bit longer than expected (approximately an extra 2–5 minutes). However, as already seen in part 1 of the study the individual variability is extremely high between the animals. In contrast to studies in sheep (Sanger and others 2011) and in vicunas (Bonacic and Macdonald 2003), body temperature and heart rate in alpacas were only very mildly, and mostly not significantly increased by restraint and shearing. In contrast to the majority of other study, we did not take any blood samples, a procedure that itself might provoke a stress response. The amount of stress caused by physical examination and measuring rectal temperature in the present cannot be assessed but the possible effect would be constant in all animals.

It has been shown that sheep can be trained for shearing accustoming them to handling and noise of the clippers (Hargreaves and Hutson 1990b). However, contradictory results have been described in a different study in sheep in which the stress response caused by shearing was even higher in animals which had been previously exposed to shearing (Mears and others 1999). To our best knowledge, such studies have not been performed in alpacas yet.

In conclusion, the standing position is the first choice for animals which appear to be tolerating the gathering, restraint and shearing well. However, in animals that cannot be restrained adequately in standing position the risk of injuries for the animal (and potentially for the assisting persons and the shearer) increases so they should preferably be shorn restrained either on a mattress on the ground or on a tilt table. Such an individual procedure seems quite feasible for most farms as the owners know the individual animals well and can mainly decide on the restraint method before shearing. Specifically designed chutes are available to restrain the alpacas for shearing, but these are currently not popular in Austria and Germany and could not be included in the study.

Acknowledgements

The authors would like to thank the alpaca farmers Eva Maria and Thomas Pötsch, Alpakaland Österreich, Tauplitz, Austria (part 1) and Kerstin and Frank Niemann, Alpacas of Density, Sonnewalde (Piesig), Germany (part 2); DVM C Kühn for observing animal protection issues (part 2), L Kawasch for technical support, Professor D Loque (Glasgow, Scotland) for his help preparing the manuscript.

Funding The study was financially supported by the Austrian Buiatric Association (Austria) and the Alpaca Association e.V. (Germany).

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Veterinary Record

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Veterinary Record 2017 180: 568 originally published online March 10, 2017
doi: 10.1136/vr.104232