

## Are alpacas especially protective of lambs? An insight into alpaca guardian behaviour towards sheep using an arena preference test

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### ABSTRACT

Alpacas are increasingly used as a guardian species in Australian sheep flocks in an effort to improve lamb survival rates. Yet little is documented about the role and mechanisms of alpaca guarding behaviours within this environment. The first phase of this study, evaluated the temperament of 44 alpacas by assessing their behaviour when individuals were separated from herd mates. Tests included agitation while in an isolation enclosure, flight speed and behaviour in observation arena tests. The second phase of the study evaluated alpaca responses to different auditory, olfactory and visual cues representative of young lambs, adult sheep (ewes) and other novel stimuli. Alpacas ( $n = 24$ ) which had not previously been exposed to sheep were individually presented with stimuli in a test arena. Behavioural responses were recorded over 2 min and analysed to compare differences between stimuli type (lamb/ewe/other) and stimuli cue form (live animal/auditory only/olfactory only/visual only). In phase one of this study alpacas exhibited little agitation while retained in an isolation enclosure with 67% of animals demonstrating a low agitation score. Females appeared to have a more 'reactive' temperament than males, exhibiting significantly faster flight speeds ( $P < 0.01$ ) and more frequent pacing behaviour ( $P < 0.01$ ) in the observational pen. In the arena test both male ( $n = 12$ ) and female ( $n = 12$ ) alpacas consistently exhibited greater attraction towards the lamb stimuli type (for all cue forms) compared to the ewe and other atypical stimuli ( $P < 0.05$  for all comparisons). Alpacas showed the greatest attraction towards the live lamb compared to visual ( $P < 0.01$ ), auditory ( $P < 0.01$ ) and olfactory lamb cues ( $P < 0.001$ ). Both male and female alpacas demonstrated similar response times and there were no significant differences between alpaca sex in the total time spent reacting to the lamb and ewe stimuli. This study demonstrates that alpacas exhibit a greater attraction towards or interest in young vulnerable animals such as lambs and supports the available anecdotal evidence that the use of alpacas as livestock guardians may be useful in improving lamb survival rates.

### 1. Introduction

Predation of livestock is a major cause of human-wildlife conflict not only in Australia but around the world (Sillero-Zubiri and Laurenson, 2001) and can incur a high economic cost for producers and welfare issues for livestock. Therefore, producers routinely invest in predator control measures such as baiting, trapping and exclusion fencing. Another strategy applied to deter predator attack is the use of guardian animals such as alpacas (*Vicugna pacos*) to protect a range of domesticated species such as sheep and poultry (Jenkins, 2003). Alpacas are domesticated South American camelids that have been primarily used for their meat, skin and fiber by Andean groups (Vilá and Arzamendia,

2022). They were introduced to Australia in the 1980's which is currently home to the world's second largest alpaca herd of 400,000, second to Peru in South America (Middleton, 2023). Free populations of camelids will generally live in family groups with a dominant male and are known to be highly social and territorial in the defend these family groups (Aba et al., 2010). Alpacas use body postures and neck postures in the defense of their territory; and communal latrines to keep members of a family within their territorial boundaries which will also deter potential contenders from other family groups (Aba et al., 2010). Alpacas exhibit a number of aggressive behaviours such as spitting, biting, kicking and high pitched vocalisations towards potential threats such as humans during handling (Windschnurer et al., 2020), and have an

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innate aversion towards canids which they will chase and attack if in the vicinity of their herd (Sumar, 1988; Aba et al., 2010). The highly social and territorial behavioural traits of alpacas, as well as their physical size and height, results in an effective display of threatening behaviours towards predators such as canids which indicate that they may be a useful guardian animal for protection of more vulnerable livestock species.

Although there are a number of studies which have reported on the demonstrated potential of alpacas to improve lamb survival rates in sheep and a deterrent to predators such as foxes (Jenkins, 2003; Mahoney and Charry, 2005), studies reporting on the efficacy and reliability of alpacas as guardian animals are limited. To better understand the factors influencing the reported success of alpacas as a guardian species, specifically toward sheep, more detailed knowledge of the mechanisms surrounding livestock guarding behaviours in this species are required, including the stimuli which may promote or trigger sheep-guarding and protection. Our recent study has shown that when alpacas co-habit with lambing ewes they appear to interact with lambs more frequently than adult sheep, suggesting a natural attraction towards lambs. (Matthews et al., 2020). While it is not known what cues might trigger alpacas to exhibit protective behaviours towards lambs, research on predator-prey dynamics, and particularly predator shape recognition, has been undertaken in prey species including fish, birds, bettongs, deer and other mammals which are able to recognise the specific shape of various predators presented as replica models (Magurran and Girling, 1986; Veen et al., 2000; Stankowich and Coss, 2007; Steindler et al., 2020). Key survival strategies for prey-type animals include recognition of conspecifics and protection of young and it is likely that shape recognition, which would enable a prey species to identify predator risk at a greater distance than olfactory or auditory detection, is equally important. Alpacas eyes are prominent and located on the side of their skulls enabling them to have exceptional peripheral vision (>330°) (Miranda-de la Lama and Villarroel, 2023). Their pupils are also horizontally oblong which has been postulated to aid surveillance against predators in an open field (Miranda-de la Lama and Villarroel, 2023). These eye characteristics may reflect the importance of shape recognition in alpacas and enhance their ability to respond to visual cues compared to other sensory cues such as sound. Alpacas have good sensitivity to sound (auditory range 40–32.8 kHz with a well-defined point of best sensitivity at 8 kHz), which is similar to cattle, goats and sheep (Heffner et al., 2014). In addition, due to their elongated necks and height advantage, it could be postulated that visual detection of predators and identification of conspecifics may be of greater survival importance compared to other sensory cues such as sound and smell.

Assessment of the role of various visual, olfactory and auditory senses in response to stimulus have previously been investigated in other ungulates using novel object tests (Anderson et al., 1999; Jones et al., 2000; Veen et al., 2000; Forkman et al., 2007). In order to examine the role of these senses, we applied a novel object test utilising visual, vocal and olfactory cues. We were particularly interested in comparing the responses of naïve alpacas (animals with no prior exposure to either sheep or lambs) to both live and model replicas of lambs and adult sheep (ewes) to test the importance of shape recognition in this species and hypothesised that alpacas would show a stronger response to visual cues than vocal or olfactory cues associated with both young and adult sheep. Our second hypothesis was that alpacas would demonstrate a stronger response towards lambs compared to adult sheep based on a previous study of alpacas cohabitating with lambing ewes (Matthews et al., 2020). As male alpacas are routinely used as guardian animals in Australian flocks (Jenkins, 2003), we also investigated the possibility of sex differences in alpaca guarding behaviours.

## 2. Materials and Methods

### 2.1. Animals ethics and welfare

Approval to conduct this experiment was granted by the University of New England (UNE) Animal Ethics Committee (AEC19–023).

### 2.2. Site and animals

This study was conducted using available stock at a commercial alpaca farm (Glenhope Alpaca Farm) located in Armidale, NSW Australia. Armidale stands 1079 m above sea level and has a long-term average rainfall of 792.4 mm. Average maximum temperature during the study was 21.5 degrees Celsius. All alpacas were born and raised on the premises and therefore habituated to the environmental conditions in which this study was conducted. Routine husbandry practices and health management of alpacas on the farm was the same as that applied to sheep including twice yearly vaccinations, routine worming, annual shearing and regular hoof and dental checks. The alpacas were maintained on improved pasture at all times with supplement feeding given as required. In the first phase of the study (beginning October 2018), temperament tests were conducted with  $n = 22$  entire male and  $n = 22$  female alpacas of which seven were 7–8 months pregnant. From this initial group, a subset of 12 male and 12 female alpacas were chosen for the second phase of testing; exposure to a novel object arena test (December 2018 through January 2019). The subset was chosen due to availability of farm animals and also to ensure we had an even number of males/females and pregnant/non-pregnant animals. Six of the 12 female alpacas were 8–9 months pregnant at the beginning of the second phase of testing. All alpacas, who ranged in age from 12 months to three years, had no previous exposure to sheep or any of the experimental stimuli and had never been previously subjected to a novel arena test. On non-testing days the alpacas remained in two herds (male and female) on the same property where the testing was conducted, under the care of the farm owner with constant supply of feed (pasture) and water.

#### 2.2.1. Habituation

Although the alpacas had previous exposure to the arena test yards as part of routine handling and management protocols, additional habituation procedures were conducted on test alpacas. The two groups (male and female) were herded separately into the arena (zone D, Fig. 1) and given 20-minutes to explore and become familiar with the surroundings of the arena, which included a small plastic step used for placement of novel stimuli during testing. This was repeated with random groups of four alpacas (given 10 min to explore) and individuals (given 5 min to explore). This habituation process ensured that the alpacas were familiar with the arena and the herding process.

### 2.3. Temperament test (Experimental Phase I)

The temperament test was conducted on the full cohort of  $n = 44$  alpacas ( $n = 22$  male and  $n = 22$  females) over one day of testing. The temperament-related measurements in this study, which have previously been used to examine temperament (Romeyer and Bouissou, 1992; Petherick et al., 2009; Dodd et al., 2014; Paredes-Sánchez et al., 2020), included agitation level when confined in a restricted space (agitation score), flight speed and observing behaviours during social isolation. The temperament tests were conducted on-farm utilising an isolation enclosure, which is generally used for confining/handling animals as part of normal management practice, and adjoining pens/yards (refer to Fig. 1 for all zones mentioned in this section). The fencing material for the holding yards and observation arena (zones A, D, E and F) were constructed of galvanized Ringlock® wire mesh while the isolation enclosure and flight zone areas (zones B and C) consisted of portable steel mesh panels (walls only). The perimeters of the observation arena were approximately  $7 \times 21$  m and the holding pen measured  $6 \times 4$  m.

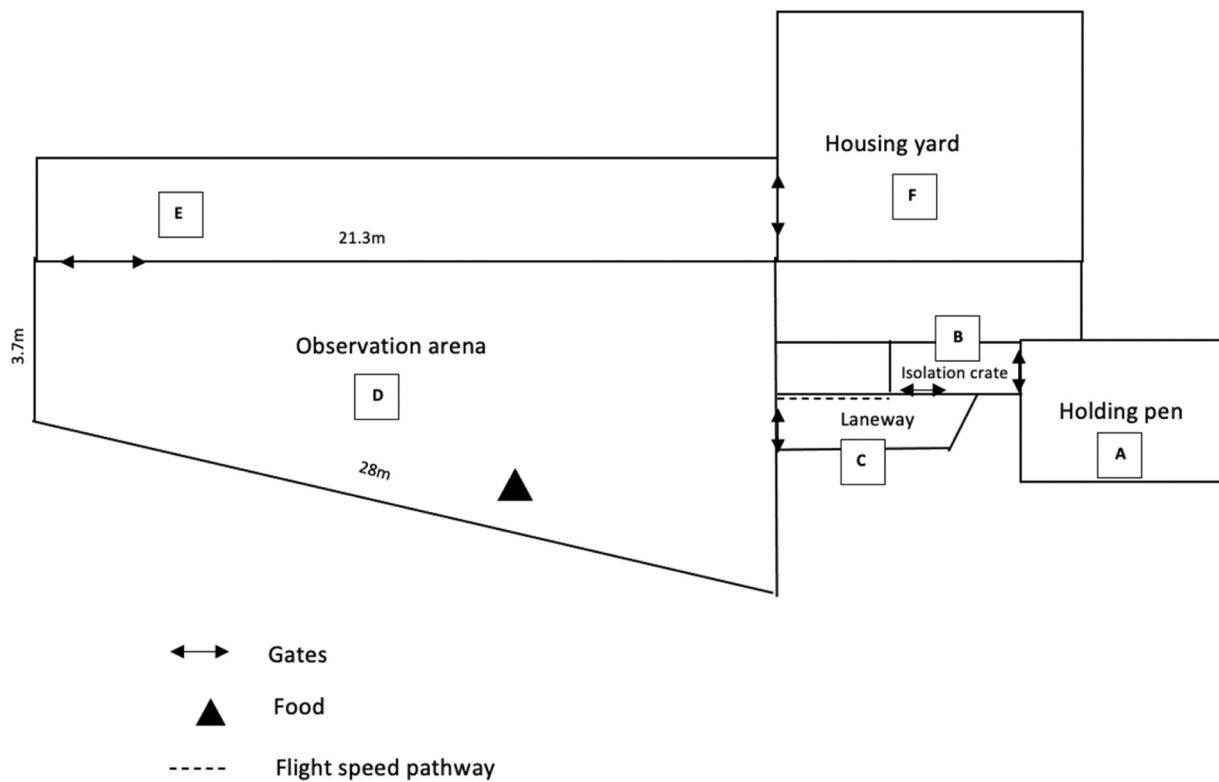


Fig. 1. Layout of testing areas. Symbols A-F indicate animal movement flow and measurement zones for temperament test. For novel object test symbols A, B, D, E and F indicate animal movement flow (isolation crate from zone B and laneway C were removed).

The sides of the isolation enclosure (2 × 1 m) were covered with shade cloth to prevent visual contact with other alpacas.

Male and female alpacas were tested on the same day in two separate groups (male or female). Animals were tested individually in a random order. Firstly, each randomly selected test animal was quietly herded into the testing area whilst the remainder of the group was retained in zone A. Each test animal was then moved into and retained in the isolation enclosure (zone B) for a total of 2 min. While in the isolation enclosure an observer assigned each alpaca a subjective agitation score from 1 to 5; (1 = calm, no movement; 2 = slightly restless; 3 = squirming, moderate signs of restlessness; 4 = continuous, very vigorous movement and 5 = twisting of the body and struggling violently) based on agitation quantification in restrained cattle (Grandin, 1993; Wemelsfelder and Farish, 2004). After 2 min in the isolation enclosure each test animal was released into a 2.8 × 1 m laneway (zone C); and a measurement of the flight time (Burrow, 1997; Petherick et al., 2009) taken to travel the length of the laneway into zone D was recorded using a FarmTek® electronic timer. Flight speed was quantified as the time (in whole seconds) recorded for an alpaca to travel between two infra-red sensors 2.8 m apart placed at each gate.

Immediately following release from the isolation enclosure each test animal was retained in the Observation arena (zone D) with hay available for a further 2 min. This period facilitated observation of other temperament related behaviours including alertness, pacing, vocalisation, defaecation and feeding behaviours/latency to feed as additional indicators of agitation level while isolated from the herd (Table 1) and were recorded with a fixed video camera. Once the observation period of 2 min was completed each test animal was herded through zone E into zone F to join other alpacas which had already been tested. This process was repeated for each alpaca. Video recordings were then analysed to determine the total time (secs) each animal spent exhibiting the various behaviours described in Table 1.

Table 1

Definition of behaviours recorded in the temperament test and novel object test arenas.

Observed behaviour	Measurement
Alert at stimulus* *	Standing: time spent standing on four legs, intently staring towards the stimulus Walking: time spent walking in a slow forward motion of more than 3 step, intently staring at stimulus
Alert, non-directional* *	Non-directional: time spent standing on four legs, tense body, ears pushed forward, staring at objects other than arena stimuli
Sniffing	Stimulus: time spent sniffing stimulus (nose comes in contact with stimulus) Ground: time spent sniffing ground (nose comes in contact with ground)
Running	Time spent running in fast forward motion of more than 3 steps
Standing	Time spent standing on four legs, ears and body relaxed
Pacing	Time spent pacing (slow forward motion of more than 3 steps with alert posture - tense body, ears pushed forward and intently staring)
Scratching	Scratches a part of body with another body part or object (e.g., scratches body with hoof)
Eating	Latency to approach and chew food (grass or hay) following release into arena
Defecating/urinating	Time spent eliminating faeces/urine
Vocalising	Frequency of vocalisations (including both high pitched sounds and quiet rumbles)
Transitions*	Frequency of change between behaviours (all observed behaviours except vocalisation)
Positioned in close proximity to stimuli* *	Time spent within a 1 m radius of stimulus

\* \*Behavioural measurement in novel object arena test only

\* Behavioural measurement in temperament test only

## 2.4. Novel object test (Experimental Phase II)

A novel object test was conducted to compare alpaca behavioural responses towards auditory, olfactory and visual (cues, both animate and inanimate), that were representative of lambs (aged 2–3 weeks), adult sheep (ewes) and other novel stimuli. For the live visual stimuli, eight ewe and lamb pairs sourced from the University of New England's experimental sheep flock were used.

### 2.4.1. Testing arena and procedures

The novel object test was conducted in the same testing area described in Section 2.3. A total of 12 male and 12 female alpacas (six pregnant and six non-pregnant), randomly selected from the temperament test flock described in Section 2.3, were individually subjected to the novel object test. Males and female alpaca were tested separately on the same day in a randomized order. One group of either 12 male, or 12 female animals, were placed in zone A and each individual test animal was quietly herded into zone B where the test animal was retained for 30 s. The test animal was then quietly herded into the testing arena (zone D) where it could observe and interact with a single stimulus. Videos of behavior exhibited in the arena (described in Table 1) were recorded with a fixed video camera over a period of 2 min while the test animal was in the testing arena. Both stimulus presentation order and alpaca test animal order were randomised to minimize effects such as learning and habituation. The period of time between each test was between 1 – 4 min while the stimuli were changed for the next test. The test alpaca was then herded through zone E into zone F, out of sight of the remaining test subjects. This process was repeated for each test animal in both male and female alpaca groups over a period of 5 h.

### 2.4.2. Stimuli

During the testing period, animals were exposed to a total of ten novel stimuli: two types of live animals (lambs and sheep), five different visual stimuli (described in Fig. 2); three different auditory stimuli (recordings of a lamb bleat, a cria (baby alpaca) bleat, and a lion's roar); and two different olfactory stimuli (lamb amniotic fluid and

commercially-sourced white vinegar). Each auditory recording was sourced from an online source and played back to the alpacas via a speaker (JBL Flip Essential 16 W). The recording of a lion's roar was used as a novel control sound which as a predator vocalisation would have differing acoustic signal qualities to that of infant calls (Riede and Fitch, 1999; Lingle et al., 2012; Lingle and Riede, 2014). The amniotic fluid was sourced from a newly born lamb and frozen until day of testing. Vinegar was used as a novel control odour and has been used in past experiments examining olfactory attraction and aversion (Semmelhack and Wang, 2009; Becher et al., 2010). A small, plastic step was placed in the test arena during both habituation and testing, under which was concealed a speaker for the auditory stimuli, or a plastic container containing the liquid scent for the olfactory stimuli. For the live animal stimulus, a pair of lambs or ewes were presented to the alpacas in a 2×2 m wire fenced enclosure within the arena. The different stimuli were presented to the animals across a total of 10 testing days and over a 6-week period to ensure breaks of at least 3 days between testing days. To reduce isolation stress on the live stimuli lambs and ewes, each set of live stimuli animals were retained in the enclosure for no more than 8 min (the duration of 2 alpaca tests) before being exchanged for another two animals. A total of eight ewe/lamb units were used as stimuli animals on a rotating basis.

## 2.5. Statistical analysis

### 2.5.1. Temperament test

An agitation score of less than, or equal to 2 was low (<5 animals) so scores 1 – 2 were grouped to represent low agitation and scores 3–5 were grouped as a category to represent moderate-high agitation. The difference in distribution of low vs moderate-high agitation score for each sex was assessed using  $\chi^2$  tests.

Video data was assessed for either determination of frequency of behaviour or cumulative time (s) spent exhibiting the behaviours described in Table 1. One animal (female) who ran out of the area, was excluded from the dataset for all analyses.

Comparison of male vs female flight speed and arena behaviour



**Fig. 2.** Live and inanimate novel arena test visual stimuli presented to alpacas. **A** live lambs and live ewes. **B** in order left to right model lamb (0.5 × 0.25 × 0.4 m), model ewe (0.9 × 0.5 × 0.7 m) and non-animal shape (footrest 0.5 × 0.5 × 0.2 m).



**Table 2**

Alpaca temperament test behaviour measurements by sex. Data are expressed as median values with range in parentheses or proportion of animals exhibiting behaviour. Significant p-values are presented in bold font. NS= $P > 0.1$ .

Temperament test behavioural measurement	Male (n = 22)	Female (n = 21 *)	Test statistics
Agitation score <sup>a</sup> [Low: high scores] <sup>^</sup>	2 (1–4) [15:7]	2 (1–4) [14:8]	$\chi^2 = 0$ , df = 1, P = NS
Flight speed (s/2.8 m)	4.59 (1.94–13.70)	2.67 (0.95–6.62)	<b>W = 447, P &lt; 0.001</b>
Time spent pacing in arena (s)	28.5 (4–79)	48 (16–105)	<b>W = 252, P &lt; 0.05</b>
Time spent alert in arena (s)	13.5 (0–64)	11 (0–57)	W = 235, P = NS
Number of arena transitions/animal	8 (2–17)	9 (2–16)	W = 284, P = 0.098
Proportion of animals running in arena (%) <sup>b</sup>	9.09 (2/22)	40.90 (9/22)	<b>OR = 0.15, P &lt; 0.05</b>
Proportion of animals sniffing in arena (%) <sup>a</sup>	54.50 (12/22)	42.86 (9/21)	$\chi^2 = 0.213$ , df = 1, P = NS
Proportion of animals vocalising in arena (%) <sup>b</sup>	22.73 (5/22)	9.52 (2/21)	OR = 0.37, P = NS
Proportion of animals defecating in arena (%) <sup>a</sup>	27.27 (6/22)	47.62 (10/21)	$\chi^2 = 1.132$ , df = 1, P = NS

<sup>b</sup> Fisher Exact test

OR = Odds ratio

\*n = 1 female ran out of arena after completion of isolation and flight speed test

<sup>^</sup> no of animals with low Agitation Score (1–2): number of animals with high Agitation Score (3–4)

<sup>a</sup> Chi-square test

(pacing, standing in alert stance and transitioning between behaviours) were evaluated by Wilcoxon one-sided tests as data were non-parametric. Difference in the proportion of male and female animals emitting any vocalisation (and also 1 vs >1 vocalisation) as well as exhibition of running, and sniffing, or defecating in the arena were tested by  $\chi^2$  tests or Fisher Exact tests where group sample size was  $n < 5$ . Classification of extreme measurements indicative of stress or agitation for some of these arena behaviours was calculated by estimation of the upper 75% quartile (high for time spent pacing/alert or transition number; low for flight speed).

### 2.5.2. Novel object arena test

One alpaca (female) ran out of the arena within the 2-minute observation period and was excluded from analyses. All other animals were included in analyses. Video data were assessed for determination of the time (s) spent by each alpaca exhibiting *total alert stance towards stimuli* behaviour (cumulative time in alert stance directed towards stimulus while either standing or walking—refer to Table 1), *alert (non-directional)*, *sniffing the stimulus or ground*, *pacing*, *running* and *scratching* behaviour during each 2-minute testing period. Video data was also analysed to determine the time (s) spent in *close proximity* (within a 1 m radius) of the stimulus by placing a transparent sheet placed over monitor screen to obscure data external to the 1 m radius.

To determine which stimuli elicited the strongest response from the alpacas, a positive response score was calculated from addition of total sum of time each alpaca spent exhibiting clear positive behaviours (total alert stance toward stimuli, sniffing the stimuli and close proximity time) towards each stimulus.

Data for each temporal variable was highly skewed so Wilcoxon Signed Rank tests (paired for each animal id) were used to compare the time alpacas spent on arena behaviours when presented with different stimuli. Behavioural data are reported as medians and ranges. Wilcoxon Signed Rank tests were also used to assess the differences in relation to alpaca sex and pregnancy status and positive response score exhibited by the alpacas to the different stimuli. Test statistics are shown for paired, one-sided Wilcoxon Signed Rank hypothesis tests unless otherwise stated. All statistical analyses were performed using RStudio 1.2.5001 software (Development Core Team, 2018).

## 3. Results

### 3.1. Alpaca temperament

The main aim of the temperament testing was to assess if alpacas exhibit differences in temperament responses while being isolated. The alpacas exhibited a relatively calm temperament while in the isolation crate with a total of 29 out of 44 animals (66%) demonstrating little or

no agitation (scores 1–2). Only 3 female (13.6%) and 2 male (9.1%) alpacas demonstrated violent twisting or struggling while confined in the enclosure (score 4). No animals demonstrated severe distress (score 5). There was no difference between male and female alpacas in relation to agitation score ( $P = 1.00$ ). We did find a difference in flight speed and pacing behaviour related to sex, as male alpacas demonstrated significantly slower flight speeds than females (4.59 vs 2.67 s,  $W = 447$ ,  $P < 0.001$ ) and spending less time pacing within the enclosed arena compared to female alpacas (28.5 vs 48 s,  $W = 252$ ,  $P < 0.05$ ) (Table 2). Female alpaca were also observed to exhibit running behaviour in the arena more frequently than males ( $OR = 0.15$ ,  $P < 0.05$ ), however there were no other differences found between male and female alpacas in relation to behaviours exhibited in the observation arena (Table 2).

Although we found some sex differences between male and female alpacas within the temperament testing, overall, the majority of animals demonstrated indicators of calm to moderate agitation behaviour when restrained (88.6%); and other arena behavioural values outside the extreme range of measurements (flight speed and transitions 77.0% of animals, time spent alert 93.2% of animals, and time spent pacing 61.4% of animals).

For the animals in the arena preference test, agitation score ranged from 1 ( $n = 3$ ), 2 ( $n = 14$ ) and 3 ( $n = 5$ ). An analysis of median flight speed, number of transitions, and time spent pacing or alert between animals with these three agitation scores levels (calm to moderate restlessness) did not reveal any significant differences between the agitation score groups so it was concluded that temperament was not a factor of concern when assessing alpaca's response to novel stimuli in the arena test.

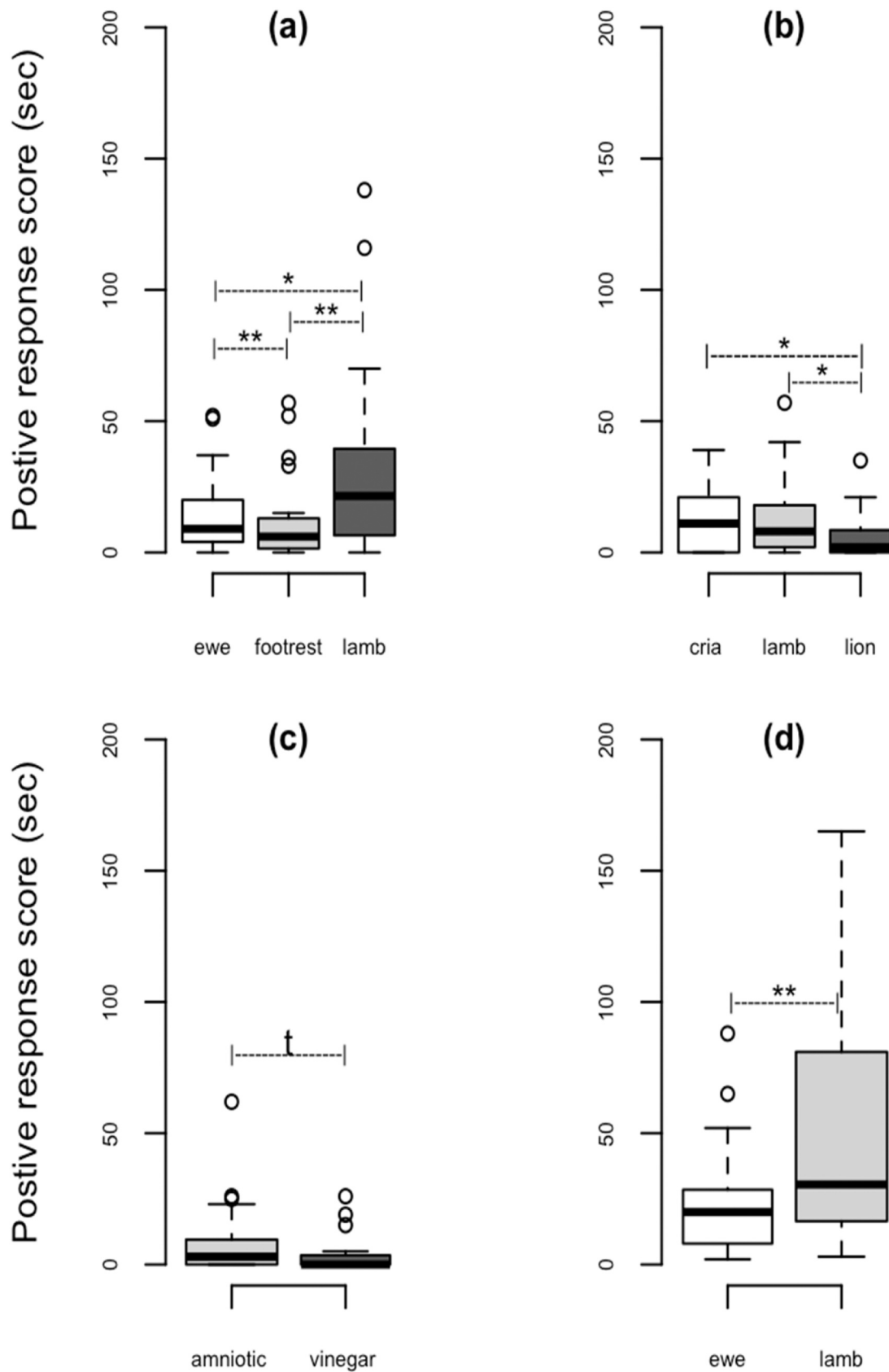
### 3.2. Alpaca preference for lamb stimuli

The alpacas exhibited a consistently significantly greater attraction towards the lamb stimuli as shown by their positive response score towards the different lamb stimuli compared to other ewe and atypical stimuli (Fig. 3). When comparing alpaca preference towards the lamb stimuli a strong preference towards the live and visual cues compared to the auditory and olfactory cues was demonstrated.

#### 3.2.1. Alpaca preference for live and visual cues

Of the 4 different forms of lamb cues which were presented to the alpaca test animals, the live cue elicited the strongest positive response score ( $P < 0.05$  for all comparisons, Fig. 4). Alpaca response towards the live animals indicated they have a significantly greater interest in the live lambs compared to live adult sheep, exhibiting significantly longer period of time spent in alert stance behaviours, sniffing and positioned in close proximity to the stimuli ( $P < 0.05$  for all behaviours, Table 3).

The visual lamb cue elicited the second strongest response with



**Fig. 3.** Positive response scores of alpacas to different (a) visual, (b) auditory, (c) olfactory and (d) live stimuli. Significant differences between stimuli type are indicated by \* =  $p < 0.05$ , \*\* =  $p < 0.01$ , t = tendency ( $0.05 < p < 0.1$ ).

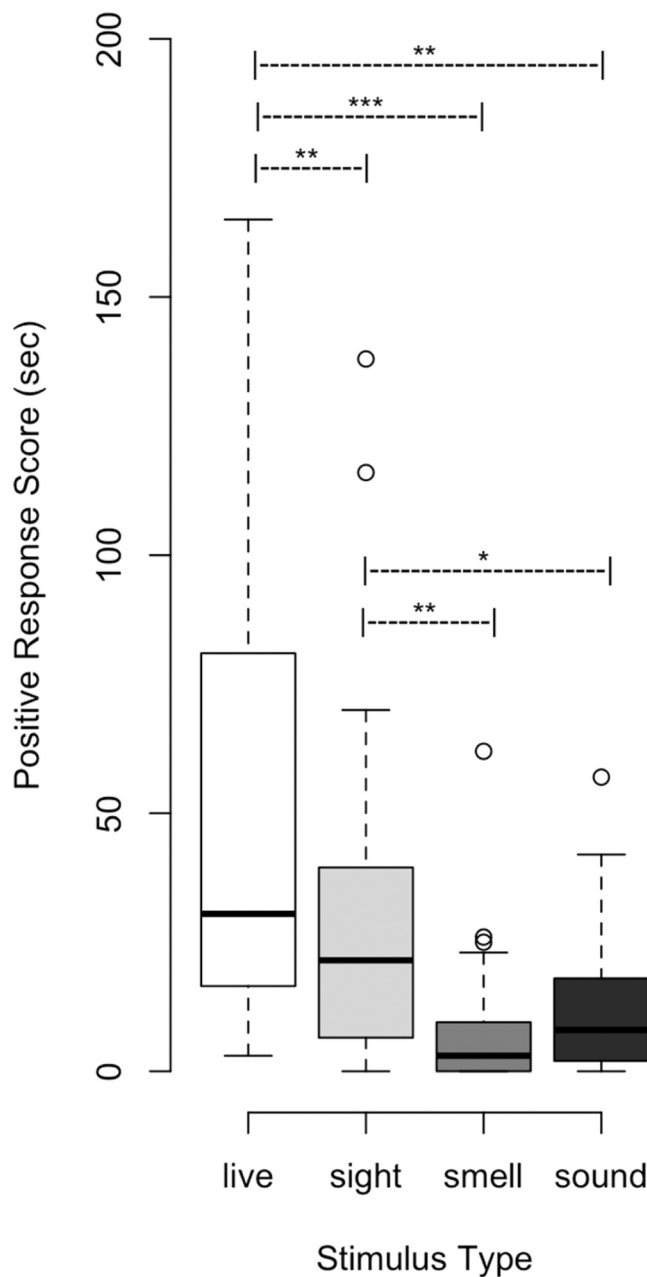


Fig. 4. Preference for lamb-related cue (live lamb, model lamb, lamb amniotic odour, lamb auditory); \* =  $p < 0.05$ , \*\* =  $p < 0.01$ , \*\*\* =  $p < 0.001$ .

Table 3

Novel object test behavioural measurements showing alpaca response times (s) to two live animal stimuli (lambs and ewes). Data are expressed as median values with range in parentheses. Significant  $p$ -values are presented in bold font. NS= $P > 0.1$ .

Arena behaviour	Live animal stimuli		Test statistics
	Lambs (n = 24)	Ewes (n = 23)	
Total alert stance towards stimuli (sum of a+b)	49 (25–80) <sup>a</sup>	32 (3–73) <sup>b</sup>	<b>V = 24, P &lt; 0.001</b>
a) Stationary alert stance	24 (0–69) <sup>a</sup>	9 (0–65) <sup>b</sup>	<b>V = 43.5, P &lt; 0.01</b>
b) Walking alert stance	24 (0–54)	14 (0–47)	V = 89 P = 0.07
Alert, non-directional	30 (0–62)	34 (0–73)	P = NS
Sniffing stimulus	0 (0–7) <sup>a</sup>	0 (0–3) <sup>b</sup>	<b>V = 6, P &lt; 0.05</b>
Sniffing ground	0 (0–7)	0 (0–14)	P = NS
Positioned within close proximity to stimulus	7 (0–113) <sup>a</sup>	3 (0–51) <sup>b</sup>	<b>V = 21.5, P &lt; 0.01</b>
Pacing	32 (9–80) <sup>a</sup>	46 (8–82) <sup>b</sup>	<b>V = 222, P &lt; 0.01</b>
Running	1 (0–10)	0 (0–11)	P = NS
Scratching	0 (0–9)	0 (0–0)	V = 0, P = 0.09

<sup>a, b</sup> Different superscripts within rows indicate medians which differ significantly ( $P < 0.05$ )

alpacas having a greater positive response score to this cue compared to the olfactory lamb cue ( $V = 237.5, P < 0.01$ ) and auditory lamb cue ( $V = 214.5, P < 0.05$ ). Alpacas demonstrated significantly less interest in the non-animal visual stimuli compared to the two animal-like visual stimuli when comparing alert behaviour towards the stimuli ( $V = 218.5, P < 0.05$ , Table 4). The alpacas spent a significantly longer total period of time in close proximity to the lamb model compared to the ewe model ( $V = 1, P < 0.01$ ) and also a longer total period of time sniffing the lamb model compared to the ewe ( $V = 28, P < 0.05$ ) and non-animal visual stimulus  $V = 28, P < 0.05$ , Table 4).

### 3.2.2. Alpaca preference for auditory and olfactory cues

Auditory and olfactory cues did not appear to elicit a strong response from the alpacas with little difference in positive response scores between these two lamb cues (Fig. 4). However, they did spend a significantly greater period of time demonstrating alert stance behaviours towards the cria auditory stimuli compared to the lion auditory stimuli ( $V = 200.5, P < 0.01$ ) and a tendency to spend longer in alert stance directed towards the lamb auditory stimuli compared to the lion ( $V = 128.5, P = 0.06$ ). The alpacas responded with similar positive response scores towards the auditory lamb stimuli vs the cria auditory stimuli ( $P = 0.15$ ) (Table 5).

Alpacas did not respond strongly towards the two olfactory stimuli as there was a low occurrence and total time spent in behaviours directed towards these stimuli (Table 6). However, the alpacas did spend more time alert at the amniotic fluid compared to the vinegar olfactory stimuli ( $V = 176.5, P < 0.001$ ).

### 3.3. Effect of alpaca sex and pregnancy status

As it was evident that the alpaca test animals had the strongest response towards the live and visual cues, we selected those specific cues to assess if there were differences in responses to stimuli associated with alpaca sex or pregnancy status (Table 7). When comparing male and female alpaca positive response scores there appeared to be a sex-related difference in response to the live ewe stimuli as females exhibited longer positive response times towards the live ewes compared to their male counterpart ( $W = 94.5, P < 0.05$ ). There did not appear to be any other significant sex-related differences regarding behavioural responses. There were no difference in response score towards the different stimuli in relation to pregnancy status. It was not known if the alpacas in this study had previously given birth to a cria, but interaction with cria and nursing would be an interesting aspect for future researchers to explore.

**Table 4**

Novel object test behavioural measurements showing alpaca response times (s) to three different inanimate model stimuli. Data are expressed as median values with range in parentheses. Significant *p*-values are presented in bold font. NS=*P* > 0.1.

Arena behaviour	Inanimate visual stimuli			Test statistics
	Lamb model (n = 24)	Ewe model (n = 23)	Atypical shape (n = 24)	
Total alert stance towards stimuli (sum of a + b below)	31 (4–70) <sup>a</sup>	23 (7–68) <sup>a</sup>	17 (5–67)	<b>Lamb vs atypical V= 218.5, P &lt; 0.05 Ewe vs atypical V= 181.5, P &lt; 0.05 Lamb vs ewe P = NS</b>
a) Stationary alert stance	13 (0–70) <sup>a</sup>	9 (0–52) <sup>a</sup>	6 (0–52)	<b>Lamb vs atypical V= 192, P &lt; 0.05 Ewe vs atypical V= 179.5, P &lt; 0.05</b> Lamb vs ewe P = NS
b) Walking alert stance	11 (0–34)	12 (0–23)	11 (2–36)	Lamb vs atypical P = NS Ewe vs atypical P = NS Lamb vs ewe P = NS
Alert, non-directional	23 (5–84)	25 (0–86)	50 (0–112)	Lamb vs atypical P = NS Ewe vs atypical P = NS Lamb vs ewe P = NS
Sniffing stimulus	0 (0–12) <sup>a</sup>	0 (0–0)	0 (0–0)	<b>Lamb vs atypical V= 28, P &lt; 0.05</b> <b>Lamb vs ewe V= 28 P &lt; 0.05</b> Ewe vs atypical P = NS
Sniffing ground	0 (0–32)	0 (0–26)	0 (0–24)	Lamb vs atypical P = NS Lamb vs ewe P = NS Ewe vs atypical P = NS
Positioned within close proximity to stimulus	0 (0–155) <sup>a</sup>	0 (0–6) <sup>b</sup>	0 (0–24) <sup>a, b</sup>	<b>Lamb vs atypical V= 55, P &lt; 0.05</b> <b>Lamb vs ewe V= 1, P &lt; 0.01</b> Ewe vs atypical P = NS
Pacing	32 (2–82)	38 (0–99)	34 (0–71)	Lamb vs atypical P = NS Lamb vs ewe V= 93.5, P = 0.09 Ewe vs atypical V= 183.5, P = 0.09
Running	1 (0–8) <sup>a</sup>	5 (0–16)	0 (0–8)	<b>Lamb vs atypical V= 60.5, P &lt; 0.05</b> <b>Lamb vs ewe V= 144, P &lt; 0.05</b> Ewe vs atypical P = NS
Scratching	0 (0–16)	0 (0–12)	0 (0–11)	Lamb vs atypical P = NS Lamb vs ewe P = NS Ewe vs atypical P = NS

<sup>a, b</sup> Different superscripts within rows indicate medians which differ significantly (*P* < 0.05)

**Table 5**

Novel object test behavioural measurements showing alpaca response times (s) to three different auditory stimuli. Data are expressed as median values with range in parentheses. Significant *p*-values are presented in bold font. NS=*P* > 0.1.

Arena behaviour	Auditory stimuli			Test statistics
	Lamb bleat (n = 23)	Cria bleat (n = 24)	Lion roar (n = 24)	
Total alert stance towards stimuli (sum of a + b below)	11 (0–61) <sup>a,b</sup>	18 (0–47) <sup>a</sup>	4 (0–35) <sup>b</sup>	Lamb vs lion V= 134, P = 0.06 Lamb vs cia P = NS <b>Cria vs lion V= 200.5, P &lt; 0.01</b> lamb vs lion V= 128.5, P = 0.09 Lamb vs cria P = NS
a) Stationary alert stance	8 (0–57) <sup>a,b</sup>	10 (0–39) <sup>a</sup>	3 (0–11) <sup>b</sup>	<b>Cria vs lion V= 145.5, P &lt; 0.05</b> Lamb vs lion P = NS Lamb vs cria P = NS
b) Walking alert stance	3 (0–10) <sup>a,b</sup>	3 (0–17) <sup>a</sup>	3 (0–35) <sup>b</sup>	<b>Cria vs lion V= 170.5, P &lt; 0.01</b> Lamb vs lion P = NS Lamb vs cria P = NS
Alert, non-directional	31 (0–95)	47 (5–89)	45 (16–106)	Cria vs lion P = NS Lamb vs lion P = NS Lamb vs cria P = NS
Sniffing stimulus	0 (0–0)	0 (0–1)	0 (0–8)	Cria vs lion P = NS Lamb vs lion P = NS Lamb vs cria P = NS
Sniffing ground	0 (0–8)	0 (0–14)	0 (0–13)	Cria vs lion P = NS Lamb vs lion P = NS Lamb vs cria P = NS
Positioned within close proximity to stimulus	0 (0–20) <sup>a</sup>	0 (0–10)	0 (0–3) <sup>b</sup>	Cria vs lion P = NS <b>Lamb vs lion V= 1.5 P &lt; 0.05</b> Lamb vs cria P = NS
Pacing	30 (6–89)	37 (16–97)	43 (4–82)	Cria vs lion P = NS Lamb vs lion P = NS Lamb vs cria P = NS
Running	3 (0–62)	0 (0–36)	3 (0–57)	Cria vs lion P = NS Lamb vs lion P = NS Lamb vs cria V= 28.5, P = 0.07
Scratching	0 (0–11)	0 (0–5)	0 (0–5)	Cria vs lion P = NS Lamb vs lion P = NS Lamb vs cria P = NS

<sup>a, b</sup> Different superscripts within rows indicate medians which differ significantly (*P* < 0.05)



**Table 6**

Novel object test behavioural measurements showing alpaca response times (s) to 2 different olfactory stimuli. Data are expressed as median values with range in parentheses. Significant *p*-values are presented in bold font. NS= $P > 0.1$ .

Arena behaviour	Olfactory stimuli		Test statistic
	Lamb amniotic fluid odour (n = 24)	Vinegar odour (n = 23)	
Total alert stance towards stimuli (sum of a + b below)	4 (0–24) <sup>a</sup>	2 (0–6) <sup>b</sup>	<b>V= 176.5, P &lt; 0.001</b>
a) Stationary alert stance	1 (0–23) <sup>a</sup>	0 (0–5) <sup>b</sup>	<b>V= 102, P &lt; 0.001</b>
a) Walking alert stance	2 (0–10)	2 (0–6)	P = NS
Alert, non-directional	46 (0–113)	36 (0–93)	P = NS
Sniffing stimulus	0 (0–15)	0 (0–18)	P = NS
Sniffing ground	6 (0–52) <sup>a</sup>	21 (0–56) <sup>b</sup>	<b>V= 45.5, P &lt; 0.05</b>
Positioned within close proximity to stimulus	0 (0–34)	0 (0–19)	P = NS
Pacing	26 (0–61)	27 (5–75)	P = NS
Running	0 (0–4) <sup>a</sup>	0 (0–2) <sup>b</sup>	<b>V= 26, P &lt; 0.05</b>
Scratching	3 (0–17)	6 (0–38)	V= 57.5, P = 0.07

<sup>a, b</sup> Different superscripts within rows indicate medians which differ significantly ( $P < 0.05$ )

**Table 7**

Comparison of alpaca positive response scores (sec) associated with sex and pregnancy status by live stimuli type (ewe or lamb) and different cue types (live and inanimate). Data are expressed as median values with range in parentheses. Significant *p*-values are presented in bold font, statistical test is paired Wilcoxon test. NS= $P > 0.1$ .

Type of cue		Sex		Test statistic *	Pregnancy status		Test statistic *
		Male (n = 12)	Female (n = 12)		Pregnant (n = 6)	Non-pregnant (n = 6)	
Live	Lamb	24 (4–162)	38 (3–165)	P = NS	38 (3–74)	79 (3–165)	P = NS
	Ewe	<b>3 (3–52)</b>	<b>10.5 (2–88)</b>	<b>W= 94.5, P &lt; 0.05</b>	29 (27–65)	16 (3–88)	P = NS
In-animate	Lamb	16.5 (4–44)	44 (0–138)	P = NS	8 (2–56)	50 (0–138)	P = NS
	Ewe	14 (0–52)	8.5 (0–51)	P = NS	21 (4–52)	9 (0–51)	P = NS

## 4. Discussion

### 4.1. Alpaca preference for lamb stimuli

Overall, we observed a consistent preference, or positive response, among both male and female alpacas towards various forms of lamb stimuli. This suggests that alpacas may be attracted to multiple lamb cues (visual, olfactory, auditory and live), which supports anecdotal evidence that alpacas running in flocks of sheep show particular interest towards lambs (Matthews et al., 2020). It is unclear as to what triggers this particular interest towards lambs or vulnerable young animals as opposed to other objects or animals as there is little research to date which explicitly explores alpaca behaviour towards lambs. However, other ungulate species have been shown to exhibit attraction toward the young of diverse species and especially towards those where vocalisation acoustic traits may be similar (Lingle and Riede, 2014). Alpacas, like other ungulates, exhibit strong maternal bonding and behaviours such as smelling neonates in order to recognise their own young, nosing to simulate suckling, vocalising and staying close to young during periods of greater vulnerability in order to protect them (Koford, 1957; Smith et al., 1994; Aba et al., 2010; Miranda-de la Lama and Villarroel, 2023). Male vicunas, the wild ancestor of alpacas, also play a major role in the defence of their offspring and spend less time grazing and more time alert as the number of females and offspring in the family increase (Vilá and Roig, 1992; Aba et al., 2010). While reports on the social behaviour of domestic alpacas are less common, it would appear that they are similar to that of the wild species (Aba et al., 2010), and indicate that the prey-species traits which enhance the survival of young have been retained. It could be theorised that lambs are similar to a newborn alpaca (cria) in terms of their appearance and therefore the alpacas could be responding with greater preference towards the lamb stimuli as the small size, shape and movement imitates a cria and initiates a similar attraction.

When comparing a lamb's bleat to a cria bleat, the alpacas responded with the same positive response towards both stimuli. Maternal-young vocalisations have been observed in alpacas with crias mimicking their mothers vocalisations, possibly to facilitate bonding (Smith et al.,

1994) and infant calls of a number of species have been shown to be of similar acoustic structure which serves to initiate a response from a caregiver (Lingle et al., 2012). The alpacas therefore may be positively responding equally to the lamb and cria bleat as have deer mothers responded to the calls of other species young where the signal frequency range is similar (Lingle and Riede, 2014). Further research on alpaca's response to other young prey animals would be beneficial to assess if they have a differential preference or attraction towards vocalisations by young animals compared adults. The alpacas did not appear to react towards the lion's roar which could be expected given that the signal is novel. Further research on alpacas' response to specific predatory stimuli such as sight, smell and sound could be valuable to expand our knowledge on other behavioural mechanisms behind livestock guarding.

### 4.2. Alpacas preference for different lamb cues

It was evident that the alpacas in our experiment demonstrated a clear preference or attraction towards the lamb stimuli, so we wanted to explore which particular type of lamb cue, or cues, elicited the greatest response or attraction. The live lamb cue elicited the strongest positive response from the alpacas which is consistent with our previous research (Matthews et al., 2020). This type of preference for stimuli that exhibit a combination of cues, such as visual and sound, compared to individual cues has also been reported in lambs which had a greater positive response towards model ewes with a vocal component compared to silent models (Walser et al., 1985). Numerous studies have demonstrated that sheep rely on a combination of senses to detect conspecifics or to distinguish their own young (Alexander and Shillito, 1985; Morgan et al., 1985) and camelids show similar maternal behaviours (Smith et al., 1994) and therefore alpacas most likely rely on a combination of cues to recognise possible conspecifics or to distinguish between animals that are non-threatening or vulnerable. This could provide insight as to why live lambs which have visual, auditory and olfactory cues including movement initiated the strongest positive response from the alpacas. The live ewe stimuli also presented a combination of visual, auditory and olfactory cues including movement, but the fact that the alpacas

demonstrated a significantly stronger positive response towards the live lambs compared to live adult sheep is an important result in this study. Such a preference or attraction towards young animals in a flock is a valuable behaviour in a guardian animal and suggests that alpacas may be more protective and stay in closer proximity to younger more vulnerable animals which are prone to predator attacks.

The visual cue (lamb model) in this study elicited the second strongest positive response from the alpacas compared to the other lamb stimuli indicating the comparative importance of this cue which has been also reported in sheep (Alexander and Shillito, 1985; Arnold et al., 1985; Walser et al., 1985; Lévy et al., 2017). Both newborn crias and lambs have similar appearance in terms of their weight (approximately 7.7 kg and 4–6 kg respectively (Fogarty et al., 1992; Bravo et al., 2009) fibre texture and silhouette, therefore it could be argued that the visual cue may have initiated a parental response from the alpacas and contributed to their attraction towards this cue.

The cues that elicited the least positive response score from the alpacas was the auditory and olfactory cues. Although vocalisation can play a role in the communication between conspecifics of gregarious species it appears that for alpacas the relative importance of these senses for attraction may be less. Little is known about alpaca's olfactory sense however their predecessor, the vicuna appears to have a relatively poor sense of smell and find it necessary to make contact with objects in their environment with their muzzle in order to obtain olfactory information (Koford, 1957). It is plausible that alpacas may also have a relatively poor sense of smell at a distance, and this may explain why a single olfactory cue did not elicit a strong response from the alpacas.

#### 4.3. Effect of temperament and alpaca sex

The main aim of conducting the temperament testing was to assess if individual alpacas exhibited significant temperament affects which would influence how the animals responded in the arena test and towards different stimuli. Alpacas are highly sociable and may have exhibited greater signs of stress during social isolation and handling by humans (Rushen et al., 1999) if they had not been habituated to the calm human handling and test arena environment of this study (Wind-schnurer et al., 2020).

When comparing alpaca female and male temperament it was found that females appeared to have a more 'reactive' temperament, based on a variety of behaviours where males demonstrated a significantly slower flight speed and less pacing/running behaviours in the observational arena compared to females. Little has been previously reported on differences in temperament between male and female alpacas, however the results in this study are consistent with other ungulate species, such as sheep, where females have been extensively reported to have a more 'reactive' temperament than male sheep hypothesised to be due to a higher fear of humans (Vandenheede and Bouissou, 1993; Viérin and Bouissou, 2003; Boissy et al., 2005) which has been correlated to the social organisation of the species (Gray and Buffery, 1971). Within species with a dominance hierarchy, the dominant sex displaying dominant behaviour have been hypothesised to be more aggressive and less fearful than the opposite sex (Vandenheede and Bouissou, 1993). The wild vicuna has family groups in which the females are ruled by a dominant male (Aréstequi-Otazú, 2005). As alpacas are closely related to the vicuna it is likely that the males could similarly share the same dominant traits which may account for lesser reactivity and fear demonstrated by the males in this study. The results of this study are also consistent with reports of female vicunas vocalising more than males while being restrained (Marcoppido et al., 2018); and wild guanaco females have also been reported to demonstrate higher cortisol levels than males during the stress of being shorn (Carmanchahi et al., 2011). Evidence based on cattle temperament suggests that overly docile females may exhibit less protective behaviour and maternal interest in young (Vetters et al., 2013). Further research on how alpaca temperament and sex correspond to guarding effectiveness would be

advantageous for a number of stakeholders including producers looking at purchasing guardian alpacas.

Although castrated males are often suggested as the preferred sex to use as guardian animals in order to avoid the management issues of entire males (Jenkins, 2003), there has been no research to date which examines the differences between entire males and females in relation to guarding effectiveness. This research is an initial step in examining the behavioural differences between entire male and female alpacas and their to livestock they would be deployed to protect. While this study found that there was no discernible difference between entire male and female alpaca response to live lambs in relation to their positive response score, other management issues such as entire male aggression and mounting behaviours would need to be considered by landholders looking to utilise these animals as flock guardians, and further studies investigating the difference between using castrated male and female alpacas could be useful.

## 5. Conclusion

The results from this study indicate that alpacas have a strong attraction and preference towards lambs compared to adult sheep (ewes) and other novel stimuli. Both male and female alpacas responded positively to all lamb cues however showed the greatest positive response towards the live lamb stimuli animal and lamb visual cues. It is unclear exactly why the alpacas have a greater preference towards the lambs than the ewes, however it could be theorised that the alpacas are responding to the lamb's small body shape, colour and size that is arguably similar to a young alpaca. This innate interest towards lambs or vulnerable young could be seen as an advantageous behaviour for a guardian animal to exhibit as they may be more inclined to stay in close proximity to young livestock prone to predator attacks. There were some differences found in relation to temperament with females being slightly more 'reactive' than males, however there was no sex difference in arena test responses to various stimuli. Further research on how differences in alpaca temperament and female vs castrated male animals influence alpaca guarding capability in the field would be beneficial for stakeholders wishing to invest in the most effective means of improving lamb survival by using guardian alpacas.

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## CRedit authorship contribution statement

PM: Contributed to the conception and design of study, acquisition of data, analyses and interpretation of data, drafting and revising the article. AD: Contributed to the conception and design of study, acquisition of data, revising article. CM: Contributed to analyses and interpretation of data and revising article. JB: Contributed to conception and design of study, acquisition of data and revising article. ED: Contributed to conception and design of study, acquisition of data and revising article. WB: Contributed to conception and design of study, acquisition of data and revising article.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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