Research

Might barking dogs alert horses to the presence of wolves?

Photo: OFB/CNRS

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Introduction

Stress responses are a crucial adaptation of prey to predation risk [1,2] and individual differences often shape these responses [3]. Despite extensive domestication favouring traits such as non-aggressive behaviour and ease of handling [4], livestock including horses may still exhibit anti-predator behaviours, for example vigilance, grouping, fighting off predators and fleeing [5]. Questions remain regarding the effectiveness of these behaviours in deterring large carnivores and in relation to their persistence and modification over time [6]. Research on this topic has focused primarily on evaluating the stress responses of animals kept in stalls or grazing in relatively small paddocks under human supervision during the day [6 – 8]. However, large carnivores are usually active at twilight or night, particularly in human-dominated landscapes [9]. Evidence of horse responses to predation risk under field conditions is scarce. Yet in some regions of Europe, free-ranging horses live in remote semi-natural landscapes with little human surveillance while exposed to predation by wolves (*Canis lupus*) [10]. To what extent horses living under such conditions have retained effective anti-predator behaviour and how this might be affected by individual differences have been insufficiently documented.

Anti-predator and stress responses, including emotional arousal, can be assessed through behavioural observations and physiological measures, such as measuring heart rate (HR) [11]. Because checking livestock regularly in the field can be logistically challenging and time-consuming, breeders often wish to track the state of their animals remotely, particularly if they are exposed to predators [12]. Few studies have evaluated the feasibility of such an approach by documenting the first necessary step, which is to quantify the stress response of livestock in the field to environmental cues signalling the presence of a predator [7].

Domestic dogs, including livestock guarding dogs (LGD), often react to the presence of predators such as wolves by persistent barking [13,14]. Dogs may bark for a variety of reasons, so this signal alone is insufficient to alert breeders or horses to the presence of wolves. However, dogs confronted with wolves might produce barks that are distinctive and unique in terms of pitch, volume and other acoustic parameters. Hunting dogs, for instance, bark differently when encountering wild boar (*Sus scrofa*) as opposed to other, less dangerous species [15]. If horses associate particular barking with the imminent arrival of a threat, namely a predator, this could provide them with a warning signal.

Although horses quickly habituate to some sounds, if dog barking is reliably associated with wolf presence and possible threat of attack then habituation is less likely to occur. A study in Germany found that horses were more alert when LGDs barked and ran to fences, suggesting that they recognised such behaviour as an indicator of potential danger [14]. Increased vigilance levels might be associated with variation in HR that could be monitored, for example using portable devices attached to livestock. Such physiological monitoring would provide breeders with valuable information about the welfare of their animals, identify which ones show leadership in dangerous situations and document chronic stress over time.

Livestock might also be sensitive to the spatio-temporal distribution of risk. Wild ungulates may, for instance, increase vigilance levels in areas of long-term risk when the immediate danger is high but not when it is low [16,17]. Prey behaviour might also vary depending on the probability of encountering predators in a given area during peak periods of activity, such as dawn and dusk for wolves [18]. Moreover, prey may benefit from a 'human shield' effect that leads to reduced predation risk near areas of high human use, which are usually avoided by predators [19,20].

Here, we report the findings of a pilot study to explore these hypotheses by experimentally quantifying the behavioural and physiological responses of horses to dog barking. We anticipated that horses exposed to this sound would show greater levels of attention and vigilance, as well as increased and more variable HR, in comparison to average conditions (i.e. in the absence of repeated dog barking). We also expected these effects to vary between individuals and to be consistently more apparent in areas of relatively greater risk (i.e. closer to wolf travel routes) than in areas of lower risk (i.e. further from such routes and closer to human dwellings).

Materials and methods

Study area and animals

We implemented our study in the pre-Alpine hills of southeast France (Figs. 1 and 2). Although most livestock damage by wolves in France is to sheep [21], equids and cattle are occasionally attacked. The habitat of the study area is essentially natural prairie delineated by rows of hedges and interspersed by blocks of exploited forest of various sizes and shapes. It is under the climatic influence of the Mediterranean, which ensures that winter temperatures are relatively mild.

We conducted trials[1] with a group of 11 pure-bred Arabian horses, including four mares aged 10 – 17 years and seven younger individuals (4 – 6 years old). They were bred to compete in international endurance races and usually sold for tens of thousands of euros. Before any interactions with wolves were suspected, these horses were kept outdoors year-round in steep and hilly terrain to develop their muscles. Winter quarters, where the horses stayed from early December to mid-April, consisted of a 23-ha pasture in the municipality of Val Buëch-Méouge (Figs. 1 and 2).

In spring 2021, the horses started displaying unusual behaviour: fleeing constantly even from their owners and showing signs of stress and exhaustion. One subsequently died and another was injured. Presence of wolves in the area was later confirmed by camera traps provided with support from local authorities (Fig. 3). Ten wolves were pictured travelling together along a trail just behind the winter quarters in November 2021. The following winter, when our experiment took place, tracks of three wolves were observed in the area as well as traces of blood in the snow suggesting that a female was reaching oestrus

[1] All the experiments undertaken in this study complied with the ethical standards of French research practices. The Val de Loire ethical committee approved the entire study (No. CE19–2023–2802–1). No procedure adversely affected the horses. In addition, the authors confirm that the study followed the guidelines of the Declaration of Helsinki.

(Fig. 3). Because of the permanent presence of wolves in the area, husbandry practices were modified to keep foals in a stable up to the age of four months and always include older, more experienced mares in the group outdoors. The horses were frequently exposed to the sound of barking dogs, including a LGD protecting sheep at a nearby farm. Three more dogs were present on the farm and there may have been others in the surrounding area.

Playback experiment

Between 21st February and 4th March 2023 we investigated the responses of horses to recorded sounds of barking dogs, specifically two Pyrenean mountain dogs (the same breed of LGD present on the neighbouring farm). Audio was extracted from a video publicly available online[2]. We used the first bark in the video to create a 30-second sequence of barking by looping it three times using Audacity 3.2.4 ®. We then prepared a 2-minute audio track[3] which was broadcast through a loudspeaker (MA 708 PACK, MIPRO, Taiwan) about 200 m from horses at two different sites within the pasture. At site 1, study horses were in a relatively open area about 300 metres away from a path frequently used by wolves. At site 2, the horses were in an area closer to a human dwelling, about 400 metres from the same trail frequently used by wolves (Fig. 4). Forage (straw) was placed at each test site to ensure that the horses stayed within the line of sight of observers when the audio track was broadcast. In the absence of forage, horses showed no apparent preference for one site or the other.

Logistical constraints prevented us from testing horse responses to control sounds (i.e. playback of sounds other than dog barking), so we refer to tests performed under standard auditory conditions (i.e. in the absence of playback) as 'quasi-controls'. In total, eight tests were performed: two playbacks and two quasi-controls at each of the two sites. Trials alternated between sites and treatments to balance replication between sites while allowing sufficient recovery time between tests to avoid carry-over effects. At site 1, playback occurred on 27th February and 3rd March, with quasi-controls on 21st and 28th February. At site 2, playback tests were on 22nd February and 1st March, with quasi-controls on 23rd February and 4th March. (A quasi-control session begun at site 2 on 2nd March was discontinued due to camera battery failure.)

Physiological data and behavioural observations

Four mares were equipped by their owners[4] with an equine heart belt containing a Polar H10 HR sensor. This had two electrodes that stayed in permanent contact with the animal's skin. A transmitter on the belt retrieved HR information from the electrodes and sent it to a mobile telephone placed in a collar around the horse's neck (Figs. 2 and 5). The horses were accustomed to handling by humans and were familiar with wearing similar belts during training and competitions. The procedure was designed to minimise stress to the horses, which came willingly to their owners and allowed the equipment to be fitted without being restrained. The heart belt was fitted at least 1 hour before the start of each observation session, enabling us to monitor HR prior to the experiment.

For each trial, observers equipped with a thermal infrared camera (Pulsar Accolade, Yukon Advanced Optics Worldwide, Lithuania) took position 100 metres from the horses at 22:00 to monitor and record their behaviour (Figs. 5 and 6). Video recording started at 22:27 and ended at 23:00. Playback was initiated at 22:30, which corresponds to a period of peak wolf activity in our study area as confirmed by camera traps. Observers tried to be discreet during trials and when leaving their positions. The HR sensor was removed by the owners at 23:30.

A naive observer[5] watched all videos and performed scan sampling to extract i) the number of horses visible in each clip; ii) their behaviour at 10-second intervals; and iii) the number of individuals exhibiting behaviours described in an equid ethogram [22] (Table 1). Although the footage did not allow for individual identification, behaviours were clearly discernible. We were particularly interested in quantifying attention and vigilance, two behaviours that are associated with the early phases of anti-predator responses to sound stimuli [8] (Table 1 and

[2] https://www.youtube.com/watch?v=H5dvvUfLJTY.

[3] In the absence of information about the average duration of dog barking in response to wolf presence in our study area, we compiled a 2-minute audio track with the following pattern: 30 seconds of barking, 10-second pause, 30 seconds of barking, 20-second pause, 30 seconds of barking. This pattern was devised to provide a sustained stimulus and provoke a gradual reaction of the horses to the potential threat.

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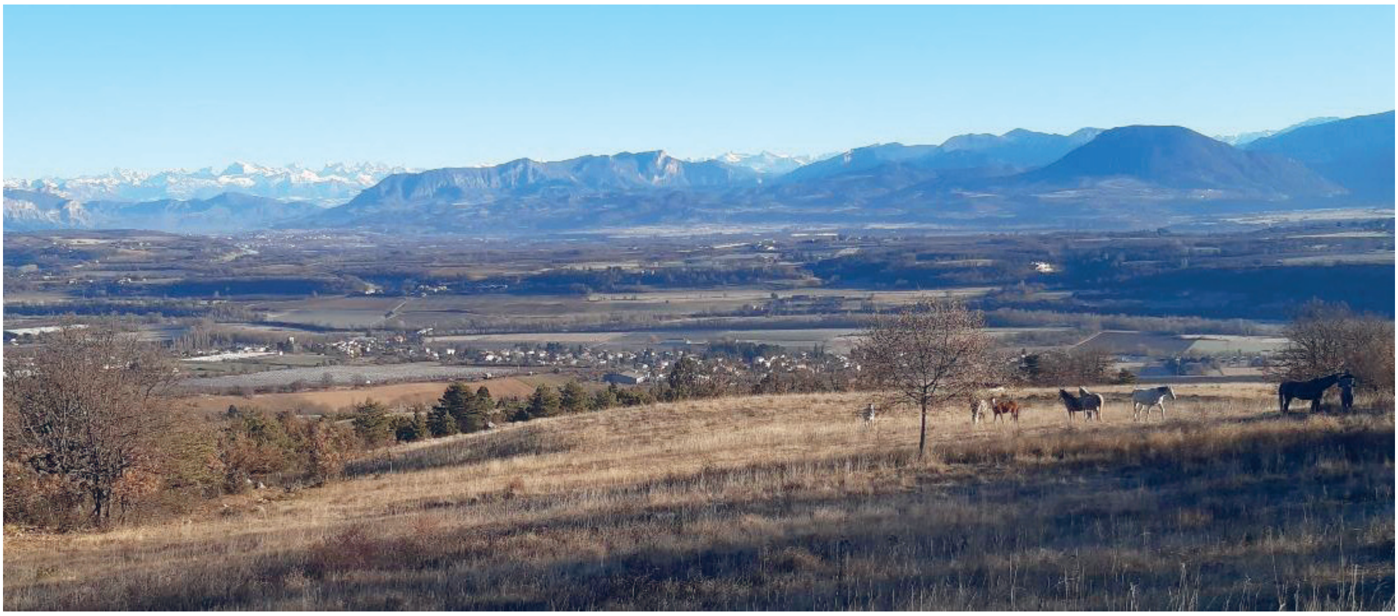

Fig. 1. Pre-Alpine landscape where trials were conducted, with some of the study horses visible in the right foreground (Photo: CNRS).

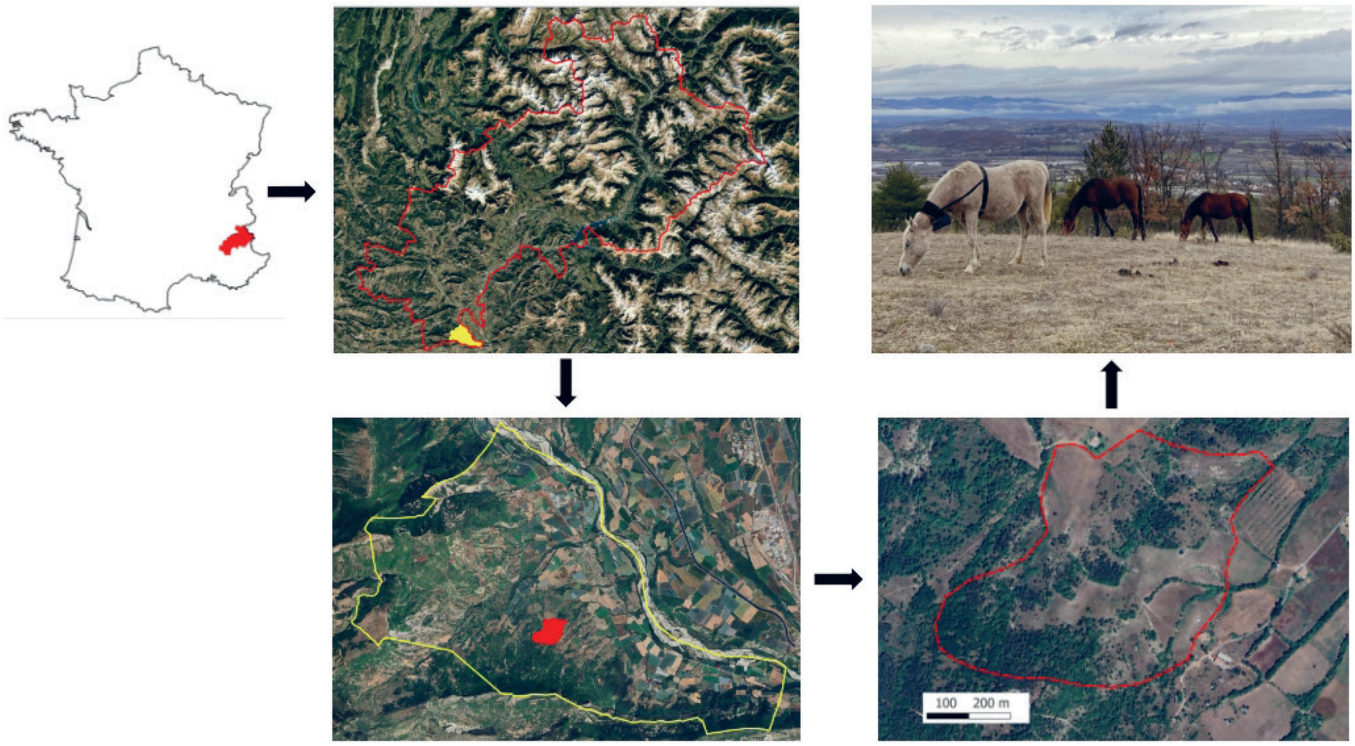

Fig. 2. Location of the study area in the pre-Alps of southeast France. The top-right image shows three of the horses including a mare wearing a collar and belt equipped with a sensor to monitor and record her heart rate (Photo: OFB).

Fig. 7). Vigilance is characterised by the individual remaining stationary and displaying strong muscle tension, an indicator of high levels of emotional arousal. A vigilant horse has its neck and tail raised, while head postures and ears are fixed, oriented toward the stimulus [23]. Attention, or monitoring, behaviour is a less intense stage in which the individual displays lower muscle tension and stands still with its neck horizontally or slightly raised. The ears, neck and head move about, slowly scanning the environment or gazing at specific stimuli, while the body is kept immobile, ready to react [24].

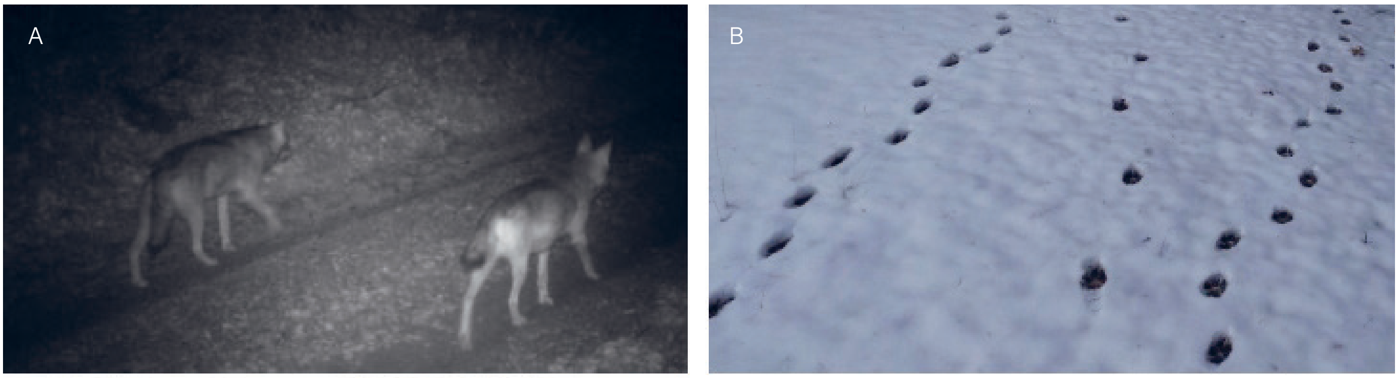

Fig. 3. Evidence of wolf presence near the study area at the time of experiments: A) two wolves travelling on a trail to the south of the study pasture; B) tracks of three wolves that crossed the study area during the winter of 2022; and C) traces of blood alongside the wolf tracks, suggesting the presence of a female wolf approaching oestrus (Photos: OFB).

Fig. 4. Schematic rendering of the set-up for playback experiments showing the positions of study horses at trial sites (1 and 2) within a 23-ha pasture in relation to a path frequently used by wolves.

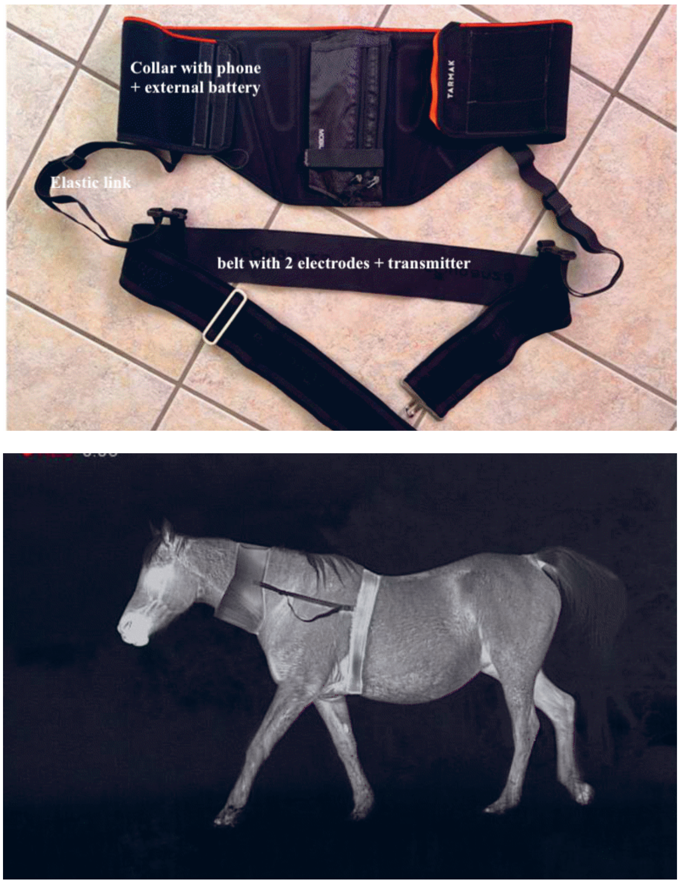

Fig. 5. Top: equine belt equipped with a Polar H10 heart rate sensor and collar containing a mobile telephone to record data. Bottom: one of the mares in the study fitted with the device, monitored via thermal infrared imagery (Photos: CNRS and OFB).

We used chi-squared tests with Yates' continuity correction and Wilcoxon signed-rank test with continuity correction to statistically assess differences in horse behaviour between playback and quasi-control conditions and between sites 1 and 2. Our datasets are available in the Zenodo repository[6].

[6] https://doi.org/10.5281/zenodo.11655717.

Table 1. Ethogram of horse behaviour (Source: based on McDonnell [22]).

Behaviour	Definition
Eating (from rack)	Eating straw with the head inside the rack.
Grazing	Feeding on grass, head down, with or without movement to search for food.
Neutral	Motionless, eyes open, muscle relaxation.
Non-directed displacement	Movement of at least three steps.
Directed displacement	Movement of at least three steps toward a stimulus.
Attention	Eyes open, neck horizontal or slightly raised, ears mobile or forward toward the stimulus, low muscle tension.
Vigilance	Eyes open, neck raised, possible snoring, ears and head forward toward the stimulus, muscle tension.
Standing up resting position	Eyes closed, feet at rest, muscle relaxation.
Sternal resting position	Eyes closed, lying down, muscle relaxation.
Negative social interaction	Threats, bites, kicks, avoidance of another individual.
Defecate	Expulsion of faecal matter.
Urinate	Expulsion of urine.
Scratching the ground	Using one of the two forelegs to scrape the surface of the ground.

Results

In the hour before each session, the four mares had a mean HR of 37.98 bpm ± 6.55, indicating stable physiological values consistent with a calm and resting state. HR tended to be higher under playback conditions, but the difference was not statistically significant when averaged per mare (Wilcoxon signed-rank test, $V = 6$, $p = 0.875$). However, we observed differences in cardiac responses between individuals (Fig. 8). Mares 1 and 2 showed a clear increase in HR under playback conditions when compared to quasi-controls, with mare 1 also displaying more variability in HR in the former case. In contrast, mares 3 and 4 showed no obvious stress response to playback conditions, their average HR even being slightly lower under playback conditions than quasi-controls.

Fig. 6. Thermal infrared imagery showing study horses before playback of dog barking. A mare equipped with a heart rate monitoring sensor is visible on the right (Photo: OFB).

Fig. 7. Thermal infrared imagery of study horses showing examples of attention (left image, one individual) and vigilance (right image, two individuals) (Photos: OFB).

Attention and vigilance events occurred more often under playback conditions (attention: N = 216, vigilance: N = 63) than during quasi-controls (attention: N = 150, vigilance: N = 0) (χ^2 = 37.92, df = 1, p < 0.001). The number of horses displaying attention peaked immediately after playback and dropped as their vigilance increased (Fig. 9). Vigilance soon subsided, with some individuals again displaying attention until the end of the 30-minute observation period. Due to small sample size (Table 2), we pooled data from playback and quasi-control to test for differences between test sites. Horses showed higher levels of attention and vigilance at site 1 closer to a path frequently used by wolves (attention: N = 216, vigilance: N = 57), than at site 2 nearer to a human dwelling (attention: N = 150, vigilance: N = 6) (χ^2 = 21.65, df = 1, p < 0.001). During the three minutes before playback began, we observed 49 events of attention and one of vigilance at site 1, as well as three events of attention at site 2 (Table 2). Nevertheless, attention and, especially, vigilance behaviours were more numerous during and following playback than before (χ^2 = 19.86, df = 1, p < 0.001).

Table 2. Number of observations of attention and vigilance among horses at two trial sites under three experimental conditions: before playback (from 22:27 to 22:30); during and after playback of dog barking (from 22:30 to 23:00); and quasi-control i.e. no playback (also from 22:30 to 23:00 but on a different day).

Site	Conditions	Attention	Vigilance
1	Before playback	49	1
	Playback	188	57
	Quasi-control	28	0
2	Before playback	3	0
	Playback	28	6
	Quasi-control	122	0

Discussion

Our pilot study corroborates other studies in concluding that Arabian horses have retained anti-predator behaviours, despite generations of domestication [6,7]. In response to broadcast sounds of dog barking, we observed a clear increase in attention and vigilance among horses kept outdoors that were familiar with the sound of LGD barking and had prior experience with wolves. These results are also in line with previous observations suggesting that horses kept with LGDs show heightened vigilance in response to barking [14]. The observed lack of vigilance in the absence of dog barking during pseudo-control periods indicates that it was, indeed, the playback that elicited the responses. Although we only included two replications at each of two sites, we did not notice any habituation (i.e. a decrease in response) to playback following the first unfamiliar bark.

The fact that vigilance and attention responses by our study horses were less frequent at the site nearer to a human dwelling is consistent with the human shield hypothesis. Our results support the idea that actual or potential human presence may reduce risk perception and stress responses in domestic animals. The horses in our study seem to have perceived areas farther from the human dwelling and closer to a path frequently used by wolves as riskier. Nevertheless, it is also possible that horses would have perceived the site closer to the path used by wolves as being riskier at night even in the absence of the house. The configuration of our study site does not allow us to disentangle the effect of the human dwelling from the effect of distance to the path on horse behaviour.

We found no statistical evidence for a change in the heart rate of study horses exposed to the playback of dog barking when compared to quasi-control conditions without playback. It should be noted, however, that this result is based on a sample size of only four mares, two of which did in fact show raised and more varied HR under playback conditions, highlighting the importance of accounting for responses at the individual level. The strong inter-individual variation observed in our study is consistent with results highlighting that individuals differ in cardiac baseline levels and physiological reactivity to environmental stimuli [11]. Such variability could be linked to position within the group hierarchy (e.g. [26]). In the context of a speed–accuracy trade-off [27], it would be more efficient for a group to follow a single, well-informed individual than trying to reach a collective decision that may take too long. In dangerous situations, the non-linear, distributed leadership common to horses can shift, with one or more animals taking the role of leaders to ensure a quick and efficient reaction [28].

From an applied perspective, remotely tracking the HR of an individual known to take the leadership role in dangerous situations might provide useful information to the

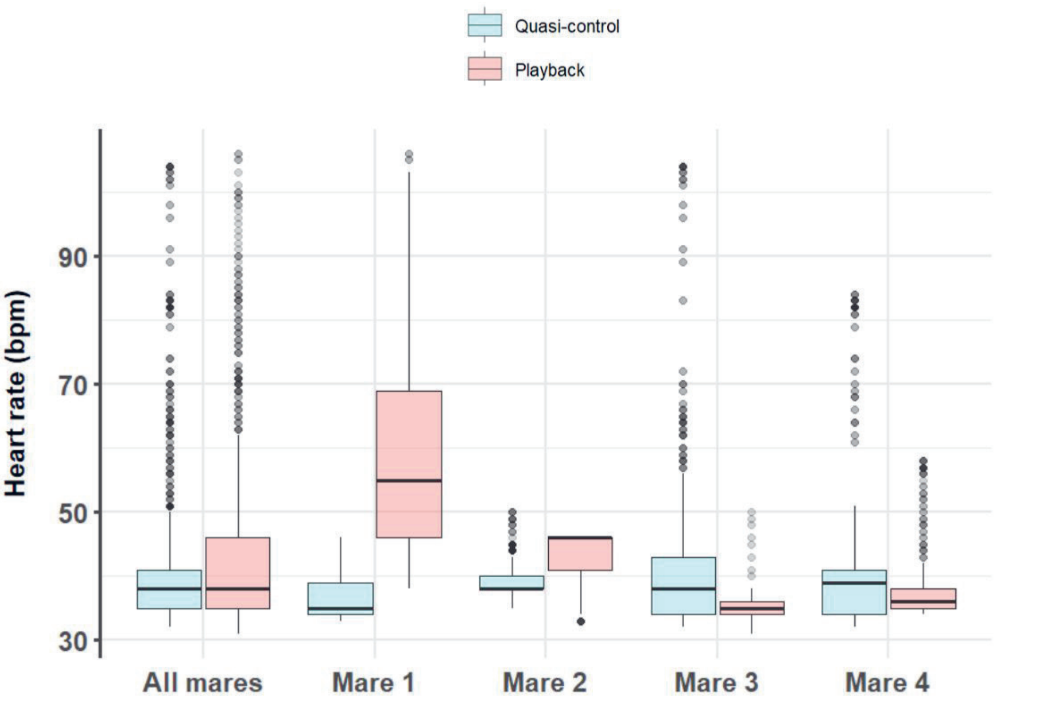

Fig. 8. Average and individual heart rate of mares under standard auditory conditions (quasi-control, i.e. no playback) and during vigilance bouts under playback of dog barking.

not be underestimated. Conversely, though speculative, a sudden peak in HR might indicate that livestock are in distress and in need of rapid human intervention. Such an application would require careful validation, since our study shows that HR may increase in response to vigilance alone, even without an actual predation threat. Still, our experimental design was based on trying to 'fool' horses into perceiving there was a risk. In any case, future work should combine HR measures with behavioural monitoring to assess the reliability of HR peaks as indicators of true risk.

breeder. The absence of both sudden peaks and high variability in HR could be indicative that there is no need to constantly worry and hurry to check on the animals, a benefit to the psychological well-being of breeders that should

Our findings are in line with other studies which found that predator-related sounds lead mainly to behavioural

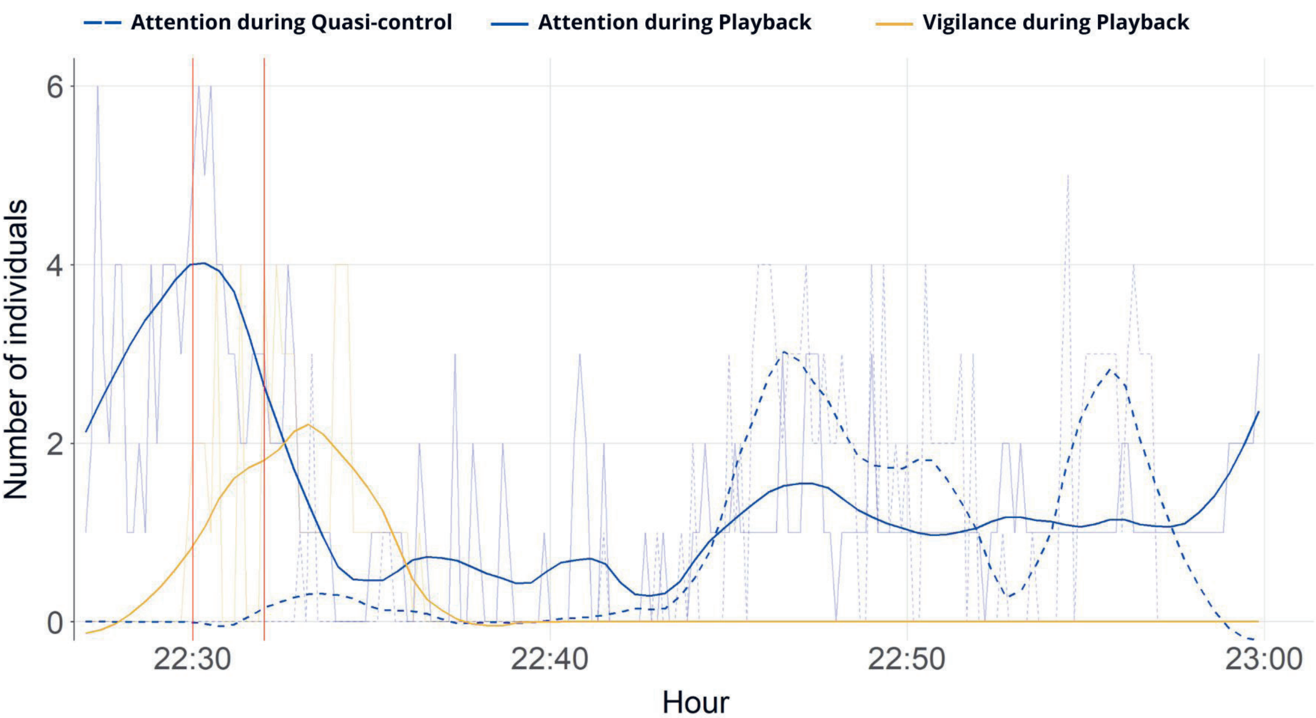

Fig. 9. Number of horses displaying attention and vigilance behaviours during quasi-controls (dashed line) and playback of dog barking (solid lines). Raw counts are shown as translucent lines with smoothed curves added to facilitate visualisation of results. The two vertical lines indicate the start and end of broadcasts during playback sessions.

changes rather than increased or more heterogeneous HR in horses [7,8]. In contrast to our study, however, the animals in these two cited studies were stabled and tested in a sandy paddock rather than a natural setting. Tests were performed during the day, when most predators are inactive. Hence, the horses faced no real predation risk, which might have led to an attenuated expression of their anti-predator behaviour. We call for further experimental studies to be conducted in field conditions and include horses that have actually interacted with wolves (a rare feat we accomplished in our study).

We acknowledge that our study suffers from several limitations besides small sample size. While we made every effort to avoid disturbing the horses, we cannot exclude the possibility that observers were the cause of attention and vigilance behaviours displayed before playback began. Perhaps the observers were more conspicuous at site 1 under playback than at site 2 and during quasi-control sessions. Other, unknown factors, such as greater attention to human activities and associated noise, might also explain horse behaviour. In any case, observer effects can strongly bias observations of animal behaviour (e.g. [25]) and should be avoided whenever possible. One solution would be to automatise the observational process to eliminate the presence of human observers altogether, although this is not without challenges if the animals are constantly moving over a wide area.

It is probable, if not certain, that the dog barking we used for playback differed in tonality from that of the neighbouring LGD. Moreover, the exact context of the barking in the video is unknown, although it seems that the LGDs in the video were barking at hikers. Ideally, we would have used a recording of the local LGD and, even better, when it was reacting to wolves. Obtaining such a soundtrack is logistically challenging and could not be achieved in time for our experiments.

Because we did not broadcast sounds other than barking, we cannot exclude the possibility that auditory stimuli unrelated to dogs or wolves would have elicited similar or even stronger behavioural responses in the study horses. Previous research has demonstrated that horses display stress responses to auditory stimuli that are not predator-related. In one study, horses reacted most strongly to unknown (non-predator) animal sounds, suggesting that their perception of threat was driven mostly by the unpredictability and novelty of the sound [8]. On the other hand, a more recent study [5] has shown horses exhibiting heightened alertness in response to deer calls and wolf howls, but not to white noise. Dogs were habitually present in our study area, which means that barking alone may not have been perceived as new or unpredictable. Yet, because we used recordings obtained from elsewhere and not from local dogs, the playback might have been perceived as novel. Future studies should strive to test for variation in the responses of horses to the sound of familiar versus unfamiliar dogs as well as investigating the contextual variability of barking, which may differ during interactions with wolves compared to other predators or stimuli that pose no threat to horses (e.g. [15]). Arabian horses seem able to differentiate between predator sounds, showing a heightened stress response to the vocalisations of relatively dangerous predators such as the wolf and leopard (*Panthera pardus*) but not to relatively innocuous ones, such as the golden jackal (*Canis aureus*) [7].

We did not conduct playback during daytime, when wolves are usually less active. Yet it would be interesting to compare the responses of horses to playback during twilight, night and day. Weaker reactions to playback during the day would lend support to our hypothesis that horses learned to associate dog barking to the proximity of wolves, as stronger responses at night and, particularly, twilight would correlate with periods of increased predator activity. Similarly, it might be instructive to repeat the experiment using horses without previous experience with wolves, which we would expect to show weaker responses to playback than animals that have learned to associate dog barking with wolf presence. Several other variables not investigated in our study might affect horse responses and hence should be considered in further experiments, including duration and loudness of the sound as well as habitat variables that could influence how horses perceive predation risk. For instance, open areas might allow earlier detection of predators, potentially facilitating flight, whereas forested areas may provide cover and thus reduce the probability of encountering a predator.

Our results do not allow us to conclude that horses have learned to associate dog barking with wolf presence. The reduced vigilance we observed near buildings may reflect a general human shield effect for horses in low-risk situations, regardless of the sound broadcast, while the increase in vigilance we documented at night father from the house may be due to greater proximity to the path used by wolves. Demonstrating a direct association between dog barks and predation risk by wolves is challenging, as it would ultimately require repeated observations of horses responding to barking in the actual presence of wolves.

Despite these limitations, our pilot study offers insights that can inform research on how domestic horses respond to predator-related cues. Future work should strive to involve multiple groups of different breeds (e.g. [6]) and with various degrees of experience with wolves and dogs in order to evaluate the generality of horse responses to predation risk and disentangle the relative influence of genetic background and experiential learning on their anti-predator responses. Investigating other physiological markers, such as glucocorticoid levels [3], could also help breeders identify individuals or groups under chronic stress, allowing timely interventions to safeguard their welfare.

Acknowledgements

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