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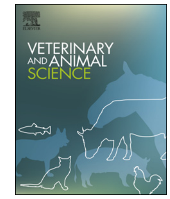

Effects of blindfolding and tail bending of Egyptian water buffaloes on their behavioural reactivity and physiological responses to pain induction

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A R T I C L E   I N F O

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A B S T R A C T

This experiment was carried out to determine the effect of blindfold and tail bend during restraint of Egyptian buffaloes on behavioural reactivity and physiological responses to stressful handling procedures. Twenty-four buffalo bulls, naïve to the testing situation, were arbitrarily assigned to either blindfold (visual restriction) (BF), tail bending (physical control) (TB) or control (CT) (no visual restriction or physical control) treatment during restraint. For three minutes each, during veterinary procedures animals entered the squeeze chute and were subjected to testing trials. Heart rate (HR), respiratory rate (RR) and various behaviours of reactivity were recorded. Average HR and RR decreased in both TB and BF bulls but the reduction was greater in BF bulls. Use of the blindfold and tail bend decreased behavioural indicators of reactivity including: chest chute forcing, head move, kicking and struggling. The reduction was greater in BF bulls in the case of use of the chest chute and struggling. Both tail bend and blindfolded buffaloes decreased behavioural and physiological indicators of stress but BF appeared more beneficial, and may therefore be recommended to reduce stress accompanying routine veterinary examination of buffaloes.

Introduction

With a population of more than 4 million animals in Egypt, buffaloes are considered an important economic source for milk and meat production and for work and draught power (FAO, 2008). Egyptian buffaloes are well adapted to subtropical environmental conditions and account for 66% of the total national production of milk and 45% of the meat (Borghese, 2010). Egyptian buffalo comes in 4th place worldwide- after India, Pakistan and China- in milk production (2,300,000 tons of milk) (FAO, 2008), and under good management their milk production ranges between 1000 and 3000 kg per lactation season, two to three times higher than that of native cows.

However, despite the large size of the Egyptian population, buffalo research has not focused on improving management and husbandry practices applied to them as much as in cattle. Improper handling of animals can injure the animal itself, the animal handler and, most importantly, the man-animal relationship. Improper handling may have also an economic impact represented as carcass damage and in the costs of animal treatment (Miranda-de la Lama et al., 2013). Therefore, proper handling of animals may not only improve human safety and animal welfare but can also have an economic impact. In addition, improving animal welfare can also result in an increase in the accuracy of experimental results that are less confounded by handling stress, and in a reduction in the number of animals used. The capacity of an individual animal to cope with environmental challenges and aversive situations is an important part of its welfare (Broom, 2008; Kilgour, Melville, & Greenwood, 2006).

In modern dairy production, buffaloes are kept loose in complex environments and exposed to potentially stressful challenges such as handling and physical restraint. Some management practices applied to large dairy animals require handling, restraint and giving them injection in squeeze chutes for routine veterinary examination and treatment, vaccination, branding, bleeding and minor surgical operations. Management practices that compromise the welfare of livestock may alter plasma hormone concentrations, induce behavioural modifications, and impair immune function (Broom & Johnson, 1993; Chirase, Greene, Graham, & Avampato, 2001; Johensen, Johannesson, & Sandøe, 2001). Behavioural and physiological responses have been proposed as indicators of the animal's capacity to cope with adverse effects of environment and can therefore be used as indexes of stress.

Numerous studies have assessed the effect on animal welfare of handling and restraint. It has been shown that handling and restraint

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can increase the heart rate, respiratory rate and plasma cortisol concentrations of cattle to levels comparable to those recorded during transport and slaughter (Herskin, Munksgaard, & Andersen, 2007; Lay et al., 1992; Mitchell et al., 2004; Solano, Galindo, Orihuela, & Galina, 2004; Stewart et al., 2013; Szenci et al., 2011; Zavy, Juniewicz, Phillips, & VonTungeln, 1992). It has also been demonstrated that the majority of cattle restrained and given injections in a head gate became highly agitated, and most animals struggled to withdraw their head or lunged forward when stimuli were applied to the neck (Baszczak et al., 2006; Ewbank, 1961; Grignard, Boivin, Boissy, & Le Neindre, 2001; Mitchell et al., 2004; Müller, Schwartzkopf-Genswein, Shah, & Von Keyserlingk, 2008). Similarly, it has been stated that cleaning of the perivaginal region and vaginal examination, procedures that involved physical handling, touching and examination of animals, has been shown to increase avoidance reactions and heart rates in the treated animals (Pilz, Fischer-Tenhagen, Grau, & Heuwieser, 2014). Attempts to escape, and physical contact with the head gate, can result in pain and injury, including bruising to the neck and back region (Grandin, 1998). Not only does increased carcass bruising represent an economic loss, but also is an indicator of compromised animal welfare (Jarvis, Messer, & Cockram, 1996).

Although an extensive amount of research work has addressed the effect of other management stressors on animal welfare, very little is known about the effect of stress of routine veterinary examination and restraint on behaviour, performance and welfare of buffaloes. Moreover, cattle rearing techniques are often used for buffaloes, even though those techniques may not be appropriate for buffaloes.

Handling and restraint of Egyptian buffaloes appears to be more difficult when compared to handling and restraint of native cows. This could be referred to the fact that buffaloes are larger in size than cattle, and that nearly all buffaloes are horned animals. However, it could also be due to the increased reactivity of the buffaloes to environmental stressors than cattle because they are less tame than cows i.e. they were domesticated at later time (5000 years) compared to cows (10,000 years) (Cockrill, 1974). Therefore, management practices that could decrease reactivity of buffaloes during handling and restraint may not only help facilitate handling these animals but could also improve their welfare.

Blindfolding has traditionally been used by ancient Egyptians to reduce reactivity of the animals, therefore facilitating the process of handling and manipulation (Shahin, 2004). The use of blindfold as a method of reducing reactivity and improving welfare of cattle by reducing levels of fear through elimination of the human proximity and handler visibility has been recommended (Ewbank, 2000; Fowler, 1995; Mitchell et al., 2004; Müller et al., 2008). However, methods other than blindfolding may be required under different conditions to restrain animals e.g. in fields where animals may require immediate treatment and handling but the use of blindfold or the restraint chute is not feasible. There is therefore the possibility of using physical method of restraint such as tail bending. However, the use of these methods in buffalo lacks scientific evidence regarding their effect on the welfare of these animals. Moreover, data on Egyptian buffalo are lacking with regard to scientific comparative studies on the effects of blindfold and tail bending on short-term behavioural and physiological measures of welfare.

The objective of the current study was to examine the potential calming effect of blindfold and tail bending on Egyptian water buffalo during restraint for routine veterinary examination. A further aim of the study was to compare between the effects of blindfolding and tail bending on the reactivity of buffaloes during restraint and pain induction.

Materials and methods

Animals

This study was carried out on a private farm, belonging to El-Gharbia Governorate, Egypt. Twenty-four Egyptian water buffalo bulls with an average body weight of 234.5 kg and an average age of 16 months were arbitrarily selected and used in this study. The herd was formed about 1 year before the experiment and had been handled regularly in a loose housing (free animals) system. All tested animals came from a single herd to reduce the potential confounding effect of previous experience.

Management

Bulls were housed in large yards (12 m width × 20 m length). Two thirds of the yard were covered with a shed and the remaining third was left uncovered. Bulls were allowed ad libitum access to green fodder (Trifolium Alexandrium), straw and fresh drinking water. A concentrate mixture was provided at a rate of 6 kg/bull/day, and was divided on two meals i.e. a meal in the morning (6 am) and a second meal in the evening (6 pm).

Experimental treatments

All animals were acclimated to an ordinary squeeze chute with a head gate for three days prior to the start of the experiment. Animals were tested daily on three consecutive days at nearly the same time each day. Each subject animal received the same treatment each day. Buffalo bulls were moved from their yards singly and were run through a straight 3 m hay rack raceway to the testing facility site. To control for the possible effect of the time of the day or the effect of communication between individual bulls being tested and those waiting in the yard, the order of testing was preassigned and counter balanced between treatments. Only one bull was inside the testing facility at a time, and the remaining animals were kept in the yard.

During restraint, the sides of the chute were adjusted to make contact with the animals body to prevent unsteady movements, but bulls were not ‘squeezed’. Once the bull was restrained in the squeeze chute, two experimenters positioned themselves, one on either side of the animal, approximately 2 m away from the animal and they remained silent and still until the data collection was finished. The experiment was conducted between 0800 and 1200 h on each of the three experimental days. Bulls were lightly restrained in the squeeze chute for 3 min (1 min pre stress induction phase, 1 min stress induction phase and 1 min post stress induction phase) and during each phase (1 min duration) data on the different measures of the study (see later) were collected. Each individual bull thus remained in the chute for three minutes testing duration before it was released.

All stock men, including those who injected the animals, operated the squeeze chute, and handled the bulls during the experiment, remained the same and maintained the same positions and conditions throughout the time of the experiment.

During the stress induction phase, three injections were given to the animal with a twenty seconds interval between injections. In each single injection the individual animal was injected with 6 ml of sterile saline solution (sodium chloride 0.9%, Al-Mottahedoon Pharma Company, 10th of Ramadan City, Egypt) subcutaneously in the side of the neck region. The reason why the animal was injected three times was to simulate what may commonly happen during the routine veterinary check where animals may be vaccinated with more than one vaccine that cannot be mixed. The volume of the medication may sometimes be large, thus necessitating dividing them into two or more injections.

Animals were arbitrarily allocated to one of the following three experimental treatments.

(1) Control treatment (CT, $n = 8$): Bulls entered into the squeeze chute and the sides of the chute were adjusted to the sides of the animal body before the procedures of stress induction were applied.
(2) Tail bending treatment (TB, $n = 8$): Bulls came into the squeeze chute and the sides of the chute were adjusted to the sides of the animal body and then the tail bending was done. The tail of the bull was held firmly close to the tail head (origin) and was then bent upward and twisted side ways, then pushed forward by one hand of the operator before the procedures of stress induction were applied. Tail bending lasted for 3 min.
(3) Blindfold treatment (BF, $n = 8$): Bulls were moved into the squeeze chute and the sides of the chute were adjusted to the sides of the animal body and then a blindfold was applied before the procedures of stress induction were applied. The blindfold procedures were carried out using a dark multilayered soft piece of cloth that covered the animal's eyes and that was fixed behind the horns using a rubber band. The blindfold was held firm in position by the application of a cotton-rope halter above it. The blindfold blocked the vision of the animals completely, and the response of the bulls to movements in their normal field of vision was absent. The application of the blindfold took approximately about 10-20 seconds.

Data collection

Sorting time

The time taken for two experienced assistants to move the animals from the assignment pen until it was entered the squeeze chute. This time was measured in seconds using a stopwatch.

Behavioural observation

Behaviour patterns recorded in this study were collected during each of the 3 min test period (pre, during and post stress induction). Behavioural patterns included chest chute forcing (leaning against the front side of the chute with the chest or forequarters), head move (moving head at any direction), struggling (lifting any leg off the ground or moving it violently), kicking (kicking the gate of the chute with the hind feet) and tail move (in case of BF and CT only). The researcher who observed and scored the behaviour stood approximately 2 meters away from the chute.

Heart and respiratory rate

Heart rate and respiratory rate were considered as indicators of the physiological response of the animal to the experimental procedures. Heart rate was recorded (count/minute) using a stethoscope as soon as the animal was positioned in the squeeze chute, and the respiratory rate was recorded (count/min) by counting the movements of the flank region before, during and after stress induction.

Flight time

The time taken by the bull from leaving the squeeze chute to reach the door of the pen was recorded as the flight time. This distance was 3 m and the time was measured using a stopwatch. Immediately, after the end of the experiment, the individual bull was released from the chute where the bull could move down a race into its original yard. As there was no close proximity to other members of the herd to attract the individual animal immediately after the release, this test represented the animal's response to the release from the chute. This time was measured in seconds using a stopwatch.

Statistical analyses

All statistical analyses were conducted using Stata version 13 (Stata Corp., College Station, TX). The association between treatments (control, tail bend, blindfold), day of testing (day1, day2, day3), and response order (pre, during and post stress) and various behavioural and physiological responses were evaluated using generalized estimating equations (GEE) with autoregressive correlation for repeated measures on animals. For RR, HR, ease of sorting and flight time, the model specification included normal distribution with identity link. The distributions of behavioural patterns responses (kicking, head move, chest-chute forcing, struggling, and tail move) were strongly right skewed, with high percentage of zeros and could not be normalized by transformation, therefore, the frequency of each behaviour was modelled using GEE with Poisson distribution and a log link. For variance estimation, the Huber/White/sandwich estimator of variance was used and variables were considered significant at $p < 0.05$. Interactions between treatment and day of testing were reported for significant terms only. Separate models were fitted for pre-stress, during stress and post-stress responses. All results are presented as estimated marginal means (EMM) ± SE.

Results

Pre stress induction

Analysis of data showed that there was an effect of the experimental treatment on the frequency of chest chute forcing, with the animals in the BF treatment showing lower frequencies compared to animals in either TB or CT. There was also an effect of the experimental treatment on the frequency of both head move and kicking, with animals in CT displaying higher frequencies compared to those in either BF or TB. There was however an experimental treatment* observation day effect on the frequency of struggling move with animals in CT displaying higher frequencies than those in both BF and TB in the second and third observation day.

There was also an experimental treatment*observation day effect on the heart rate with animals in the CT showing higher counts compared to those in either BF or TB in the second observation day, and those in the both CT and TB showing higher counts than those in BF in the third observation day. For tail movements and respiratory rate, the results were non-significant. Data of pre stress induction phase are presented in

Table 1
Average physiological and behavioural parameters of bulls in the three experimental treatments during pre stress induction phase. NS = non significant. Means within the same row with different superscripts are significantly different. Data presented as means ± SE.

Treatment					
Parameter		Control treatment (CT)	Tail bend (TB)	Blindfold (BF)	p value
Heart rate frequency	Day 2	$71.75^a \pm 1.57$	$63.75^b \pm 2.72$	$57.00^b \pm 2.64$	< 0.001
	Day 3	$67.75^a \pm 1.02$	$64.75^a \pm 2.68$	$55.00^b \pm 1.08$	< 0.001
Respiratory rate frequency		18.08 ± 0.74	18.54 ± 1.07	17.33 ± 0.58	NS
Chest chute forcing frequency		$1.67^a \pm 0.35$	$0.63^b \pm 0.15$	$0.45^b \pm 0.16$	< 0.01
Head move frequency		$2.25^a \pm 0.28$	$0.33^b \pm 0.12$	$0.25^b \pm 0.09$	< 0.001
Struggling move frequency	Day 2	$1.13^a \pm 0.21$	$0.13^b \pm 0.10$	$0.13^b \pm 0.10$	< 0.001
	Day 3	$1.50^a \pm 0.47$	$0.13^b \pm 0.11$	$0.25^b \pm 0.14$	< 0.001
Kicking frequency		$0.92^a \pm 0.20$	$0.54^b \pm 0.12$	$0.29^b \pm 0.11$	< 0.05
Tail move frequency		3.25 ± 0.44	————	2.71 ± 0.40	NS

Table 2
Average duration sorting time (second) by the bulls in the three experimental treatments in pre stress induction phase. Means within the same row with different superscripts are significantly different. Data presented as means ± SE.

Experimental day				
Parameter	Day 1	Day 2	Day 3	*p* value
Sorting time duration (second)	51.87[a] ± 1.85	48.50[b] ± 1.99	43.42[b] ± 1.94	< 0.001

Table 1.

Average duration of sorting time showed only an effect of the observation day with animals in all experimental groups requiring lower ease of sorting time in the third observation day compared to the first observation day (Table 2).

During stress induction

Average frequency chest chute forcing and head move showed an effect of experimental treatment with animals in CT displaying higher frequencies compared to their counterparts in both BF and TB. Average frequency struggling move showed also an effect of experimental treatments with animals in CT displaying higher values than those in either BF or TB, and those in TB displaying also higher frequencies compared to those in BF. Similarly, average frequency tail move showed an effect of experimental treatment with animals in CT displaying higher values compared to those in BF. Average kicking frequency showed an experimental treatment*observation day effect increasing in CT animals compared to their conspecifics in either BF or TT in both the second and third observation day.

Average respiratory rate frequency showed an effect of experimental treatment with animals in BF displaying lower counts than those in either TB or CT, and those in TB displaying lower counts relative to their counterparts in CT group. There was also an effect to the experimental treatment on the average heart rate frequency with animals in both BF and TB displaying lower counts compared to those in the CT group in the first observation day. Whereas in both the second and third observation day, animals in the BF displayed lower counts relative to those in either TB or CT group, and animals in TB displayed lower counts relative to those in CT group. Data of during stress induction phase are presented in Table 3.

Post stress induction

There was an effect of the experimental treatment on the frequency of both head move and struggling move with bulls in both BF and TB displaying lower head move frequency compared to those in CT, and those in BF displaying lower struggling move frequency relative to their conspecifics in either TB or CT group.

There was also an effect of the experimental treatment on the respiratory rate frequency with animals in both BF and TB having lower frequency compared to those in CT group. However, average frequency heart rate showed an experimental treatment / observation day effect with animals in both BF and TT having lower frequencies relative to those in CT in the first and second observation day, and those in BF having lower frequency compared to those in TB in the third observation day.

Analysis of results showed that there was an effect of the experimental treatment on the flight time duration with bulls of the BF showing longer flight times compared to those in both TB and CT, and those in TB showing longer flight time duration compared to those in CT group. For chest chute forcing, kicking movements and tail movements, the results were non-significant. Data of post stress induction phase are presented in Table 4.

Discussion

The results of this experiment showed that both blindfolding and tail bending of Egyptian buffaloes may be beneficial in reducing behavioural and physiological stress responses during restraint for routine veterinary examination. The reduction of stress indicators was present during the whole 3 min of the restraint, including before, in between and after the three injections. Nevertheless, the effects seemed to be strongest in between the injections with all 5 behavioural and 2 physiological indicators of stress being reduced, while in the first and last minute of the restraint, some of the indicators (e.g., kicking, tail movements, respiration rate) were not significantly different between the three treatments. These findings indicate that both blindfolding and tail bending reduce the stress of restraint, but may be even more effective in reducing the combined restraint and pain stress.

The reduction in stress responses in blindfolded buffaloes observed in the current study could be due to the reduced level of fear as a result of visual restriction in these animals. It has been reported that blindfolding beef heifers during routine invasive procedures induced a reduction of 43.39 % in their behavioural (struggling and movement) and a reduction of 15.4 % in their physiological (heart rate) responses to restraint (Mitchell et al., 2004). The possibility that blindfolding animals eliminates visual communication between animals and their environment (both animate and inanimate) and therefore renders them calmer has been raised (Fowler, 1995). It has been shown that eliminating sensory visual inputs in adult Brahman cattle during restraint using a mask, decreased their emotional reactivity (Andrade, Orihuela, Solano, & Galina, 2001). It has also been demonstrated that covering

Table 3
Average physiological and behavioural parameters of bulls in the three experimental treatments in stress induction phase. Means within the same row with different superscripts are significantly different. Data presented as means ± SE.

Treatment					
Parameter		Control treatment (CT)	Tail bend (TB)	Blindfold (BF)	*p* value
Heart rate frequency	Day 1	91.63[a] ± 1.57	68.25[b] ± 2.72	66.85[b] ± 2.64	< 0.001
	Day 2	92.23[a] ± 2.22	67.25[b] ± 2.80	60.15[c] ± 0.83	< 0.001
	Day 3	87.75[a] ± 1.34	68.00[b] ± 2.87	57.00[c] ± 0.72	< 0.001
Respiratory rate frequency	Day 1	31.63[a] ± 1.49	23.88[b] ± 0.86	20.63[b] ± 0.58	< 0.001
	Day 2	31.38[a] ± 1.46	26.25[b] ± 0.52	19.50[c] ± 0.36	< 0.001
Chest chute forcing frequency		2.83[a] ± 0.51	0.25[b] ± 0.16	0.41[b] ± 0.14	< 0.001
Head move frequency		2.50[a] ± 0.28	0.33[b] ± 0.11	0.25[b] ± 0.08	< 0.001
Struggling move frequency		2.00[a] ± 0.06	0.85[b] ± 0.04	0.33[c] ± 0.02	< 0.001
Kicking frequency	Day 2	1.63[a] ± 0.25	0.37[b] ± 0.17	0.50[b] ± 0.25	< 0.05
	Day 3	2.50[a] ± 0.37	0.50[b] ± 0.24	0.75[b] ± 0.22	< 0.001
Tail move frequency		6.80[a] ± 1.02	————	3.29[b] ± 0.44	< 0.001

Table 4
Average physiological and behavioural parameters of bulls in the three experimental treatments in post stress induction phase. NS = non significant. Means within the same row with different superscripts are significantly different. Data presented as Means ± SE.

Treatment					
Parameter		Control treatment (CT)	Tail bend (TB)	Blindfold (BF)	*p* value
Heart rate frequency	Day 1	$79.13^{a} \pm 2.23$	$67.00^{b} \pm 3.06$	$64.5^{b} \pm 1.53$	< 0.001
	Day 2	$76.50^{a} \pm 2.10$	$63.50^{b} \pm 2.38$	$57.75^{b} \pm 1.26$	< 0.001
	Day 3	$57.75^{ab} \pm 2.98$	$67.25^{a} \pm 3.41$	$55.00^{b} \pm 1.08$	< 0.001
Respiratory rate frequency	Day 2	$23.50^{a} \pm 0.74$	$21.00^{b} \pm 0.90$	$18.50^{b} \pm 0.66$	< 0.001
Chest chute forcing frequency		0.29 ± 0.19	0.16 ± 0.13	0.13 ± 0.12	NS
Head move frequency		$1.54^{a} \pm 0.24$	$0.22^{b} \pm 0.14$	$0.21^{b} \pm 0.13$	< 0.001
Struggling move frequency		$0.41^{a} \pm 0.18$	$0.29^{a} \pm 0.15$	$0.12^{b} \pm 0.09$	< 0.05
Kicking frequency		$0.63^{a} \pm 0.11$	$0.33^{b} \pm 0.09$	$0.25^{b} \pm 0.09$	< 0.05
Tail move frequency		1.85 ± 0.30	————	1.80 ± 0.28	NS
Flight time duration (minute)		$1.63^{c} \pm 0.05$	$2.24^{b} \pm 0.08$	$3.01^{a} \pm 0.05$	< 0.001

broilers' heads with a hood before they were shackled, substantially reduced the time spent struggling and the numbers of struggling bouts and vocalizations (Jones & Satterlee, 1997).

Restriction of vision through the reduction of ambient light intensity has been demonstrated to make non-human animals calmer during restraint or capture, including domestic chicken (Jones, 1986; Jones, Hagedorn, & Satterlee, 1998), red deer (Haigh & Friesen, 1995; Pollard & Littlejohn, 1994) and squirrels (Mantor, Krause, & Hart, 2014). However, the present study could not demonstrate whether the blindfolded buffaloes were calmer because the blindfold eliminated the ability of animals to detect the presence of humans in close proximity, or all visual inputs (presence of human and other environmental variables). However, bearing in mind that chickens, deer, squirrels and buffaloes are prey species, it appears that eliminating vision in these species may impair their ability to assess the environment. It has been shown that removing distractions such as shadows, reflections and people from the visual field of livestock species facilitate their movement and reduce balking and 'stops' (Grandin & Johnson, 2005; Grandin, 2007).

There is also the possibility that, in the current study, blindfolded animals displayed lower levels of behavioural and physiological indicators of stress because they were more fearful. Tonic immobility, as a reaction of an animal to fear, is characterized by a catatonic-like state of reduced responsiveness to external stimulation has been documented in other species such as domestic fowl (Forkman, Boissy, Meunier-Salauen, Canali, & Jones, 2007; Jones, 1986). However, this possibility can be ruled out by the findings that the proportion of cows that were immobile in fear provoking situations was very low (Dantzer, Mormede, Bluthe, & Soissons, 1983; Kilgour, 1975).

Measured stress responses in the current study were also reduced through the tail bending treatment. The effect of tail bending on calming of cattle was mentioned by Ewbank (2000, page 98) and the method is occasionally recommended in textbooks on animal handling (e.g. Chastain, 2017). Our study is the first to examine experimentally the behavioural and physiological effects of this method in any domestic bovid species, except for the small-scale unpublished study by Woodley (2007) on calves. The mechanism by which tail bending induces lower stress reactions cannot be determined from the results of this study. Two possible mechanisms were proposed for the similar procedures of nose twitching and ear twitching in horses. Nose twitching, if applied for a maximum five minutes, achieves behavioural calming through an endorphin-mediated analgesic effect (Lagerweij, Nelis, Wiegant, & Van Ree, 1984). Nose twitching is apparently not stressful or aversive for horses as it decreases heart rate and does not create attempts to avoid it when it is applied for a second time (Flakoll, Ali, & Saab, 2017). On the contrary, ear twitching in horses is likely aversive and immobilizes horses through pain and/or fear as it results in increased heart rate and salivary cortisol levels (Flakoll et al., 2017). Further research on tail bending in bovidae is needed to determine the mechanisms of its calming effect. Nevertheless, the sorting time in our study was decreased over days and there was no interaction between treatment and day. This might indicate that the bulls in the tail bending treatment were not experiencing significant pain and/or fear, as that would likely make them reluctant to enter the chute. However, further studies might be needed to investigate the effects of tail bending in buffaloes, including whether this procedure induces endorphins release, or acts through different mechanisms, in order to see if tail bending has the same effect as twitches in horses.

Therefore, the findings of the current study indicate that both blindfolding and tail bending of buffalo bulls may reduce the combined effects of restraint and pain stress. However, blindfold may be more effective and more preferable than tail bend in reducing stress and pain accompanying routine veterinary examination of buffaloes. One reason for this could be the fact that blindfolding appeared to have more influence on physiological measures such as respiratory and heart rates, and behavioural measures of stress including struggling and flight time than tail bending. Another reason to prefer blindfold over tail bend is that it restricts the vision of the animal through the reduction of ambient light intensity, and requires shorter and lower direct physical handling of animals and therefore could make the animal more calmer. In addition, the application of the tail bend procedures could be directly stressful in its own right.

It should finally be acknowledged that as the samples size of the study is small the results must be regarded with caution.

Conclusion

We conclude that, both tail bend and blindfolding of water buffaloes showed lower behavioural and physiological indicators of stress responses, but that blindfolding appeared more beneficial and may therefore be recommended to reduce stress accompanying routine veterinary examination, to facilitate handling ease of buffaloes and to improve their welfare. This improvement in the welfare of buffalo bulls is potentially beneficial from both the scientific and economic perspectives and also for the sake of public considerations. The reduction in the stress responses may also be of particular importance to affirm the safety of both the animal and the handler when restraining buffaloes for routine veterinary examination and treatment.

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