Special Research Report

Impact of previous exposure to wolves on temperament and physiological responses of beef cattle following a simulated wolf encounter [1]

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Synopsis

The simulated wolf encounter increased excitability and fear-related stress responses in cows previously exposed to wolves, but not in cows unfamiliar with this predator.

Summary

One hundred multiparous, pregnant, non-lactating crossbreed beef cows from the Eastern OR Agricultural Research Center - Burns (**CON**; n = 50) and from a commercial operation (**WLF**; Council, ID, n = 50) were assigned to the experiment. However, CON cows were unfamiliar with wolves, whereas WLF cows belonged to a herd that experienced multiple confirmed wolf predation episodes. On d 0, CON and WLF cows were ranked by temperament, BW, and BCS, and allocated to 5 groups (10 CON and 10 WLF cows/group). Groups were individually subjected to the experimental procedures on d 2 (n = 3) and d 3 (n = 2). Within each group, cows were evaluated for temperament, a blood sample was collected, and a data logger was inserted intravaginally to record body temperature at 30 s intervals (pre-exposure assessment). After these assessments, cows were sorted by previous wolf exposure, moved to 2 adjacent drylot pens (10 WLF and 10 CON cows/pen) and subjected to the simulated wolf encounter for 20-min, which consisted of: 1) cotton plugs saturated with wolf urine attached to the drylot fence, 2) continuous reproduction of wolf howls, and 3) 3 trained dogs walked using a leash outside the drylot perimeter fence. Thereafter, WLF and CON cows were commingled and returned to the handling facility for removal of data loggers, temperament evaluation, and blood collection (post-exposure assessment). However, cotton plugs saturated with wolf urine were attached to the handling facility, wolf howls were reproduced during processing, and cows were also exposed for 20 s to the 3 dogs while restrained in the squeeze chute, but immediately before post-exposure assessments. Chute score, temperament score, and plasma cortisol concentration increased ($P \leq 0.01$) from pre- to post-exposure assessment in WLF, but did not change in CON cows ($P \geq 0.19$). Exit velocity decreased ($P = 0.01$) from pre- to post-exposure assessment in CON, but did not change ($P = 0.97$) in WLF cows. In addition, WLF cows had a greater ($P = 0.03$) increase in temperature from pre- to post-exposure assessments compared with CON cows. In conclusion, the simulated wolf encounter increased excitability and fear-related physiological stress responses in cows previously exposed to wolves, but not in cows unfamiliar with this predator.

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Introduction

The reintroduction of grey wolves into the Yellowstone National Park allowed wolf packs to disperse into regions outside the Greater Yellowstone Ecosystem, including agricultural lands in Idaho and Oregon (Larsen and Ripple, 2006). As a result, wolves started to inhabit and hunt in livestock grazing areas, which increased the incidence of cattle predation by wolves in both states (Idaho Department of Fish and Game and Nez Perce Tribe, 2013; Oregon Department of Fish and Wildlife, 2013a). The economic and productive implications of predators on livestock systems is often evaluated based on the number of animals injured or killed (Treves et al., 2002; Oakleaf et al., 2003; Breck and Meier, 2004); however, these parameters may not be the only negative impacts that wolf predation causes to beef cattle systems (Kluever et al., 2008; Laporte et al., 2010).

The mere presence of predators alters stress physiology and behavior parameters of the prey (Creel and Christianson, 2008), particularly if the preyed animal was already subjected to similar predation episodes (Boonstra, 2013). More specifically, fear of predation may alter cattle temperament and stimulate adrenal corticoid synthesis (Laporte et al., 2010; Boonstra, 2013), which have been shown to negatively impact health, productive, and reproductive parameters in beef cattle (Cooke et al., 2009; Cooke et al., 2012; Francisco et al., 2012). Based on this rationale, we hypothesized that wolf presence near cattle herds stimulates behavioral and physiological stress responses detrimental to cattle productivity and welfare, particularly in cattle from herds previously predated by wolves. Hence, the objective of this experiment was to evaluate temperament, body temperature, and plasma concentration of cortisol in beef cows previously exposed or not to wolves, and subjected to auditory, olfactory, and visual stimuli designed to simulate an encounter with wolves.

Materials and Methods

This experiment was conducted at the Oregon State University – Eastern Oregon Agricultural Research Center (EOARC; Burns, OR). Animals utilized were cared for in accordance with acceptable practices and experimental protocols reviewed and approved by the Oregon State University, Institutional Animal Care and Use Committee.

Animals and diets

This experiment was conducted using 100 multiparous, pregnant, non-lactating crossbred beef cows from the EOARC Burns (**CON**; n = 50) and from a commercial cow-calf operation (**WLF**; located in Council, ID, n = 50). Both locations occasionally used domestic herding dogs to move cattle across pastures or to the handling facility. The CON cows (age = 5.0 ± 0.1 yr, BW = 1150 ± 13 lbs, BCS = 4.80 ± 0.04, and approximately 6 mo in gestation at the beginning of the experiment) were randomly selected from the EOARC Burns mature cowherd, which is reared and maintained in areas (Burns and Riley, OR) without known wolf establishment or predation episodes (Oregon Department of Fish and Wildlife, 2013a). Hence, CON cows were unfamiliar with wolf presence and predation. The WLF cows (age = 4 yr; BW = 1128 ± 15 lbs; BCS = 4.90 ± 0.06, and approximately 6 mo in gestation at the beginning of the experiment) were randomly selected from the commercial operation, which is located in an area (Council, ID) with established wolf packs (McCall-Weiser Wolf Management Zone; Idaho Department of Fish and Game and Nez Perce Tribe, 2013). Further, WLF cows belonged to a herd that experienced multiple confirmed wolf predation episodes from 2008 to 2012 (USDA-APHIS, Idaho Wildlife Services, Boise, ID; confirmation documents available upon request to corresponding author), although none of the WLF cows were directly predated or injured by wolves. Therefore, WLF cows were considered familiar with wolf presence and predation episodes.

The WLF cows were transported to the EOARC Burns 50 d prior to the beginning of the experiment (d 0). During this period (d -50 to d 0), CON and WLF cows were commingled and maintained in a single meadow foxtail dominated pasture harvested for hay the previous summer, and had ad libitum access to meadow-grass hay, water, and a vitamin-mineral mix (Cattleman's Choice, Performix Nutrition Systems, Nampa, ID). Cows were also individually processed through the EOARC handling facility, but not restrained in the squeeze chute, once a week from d 50 to -2 to acclimate WLF cows to the EOARC personnel and facilities (Cooke et al., 2012).

On d 0, CON and WLF cows were ranked by temperament score (by the same single technician), BW, and BCS, and allocated to 5 groups of 20 cows each (10 CON and 10 WLF cows per group). Each group of 20 cows was maintained on individual meadow foxtail pastures harvested for hay the previous summer during the experimental period (d 0 to 3), and had ad libitum access to water and the previously described meadow-grass hay and vitamin-mineral mix.

Simulated Wolf Encounter

Due to daylight limitations, 3 groups were randomly selected and received the experimental procedures on d 2, whereas the remaining 2 groups received the experimental procedures on d 3. While an individual group was being subjected to the simulated wolf encounter at the EAORC handling facilities, the other groups remained on their respective pastures. Groups were maintained on pastures that were ≥ 0.3 miles distant from the handling facilities to prevent that cows perceived the simulated wolf encounter model while on pasture.

Pre-exposure assessments. The evaluated group was gathered in its respective pasture and walked to the handling facility, where cows were evaluated for temperament (chute score, exit velocity, and temperament score, by the same single technician; Cooke et al., 2012). Immediately after chute score evaluation, a blood sample was collected and a HOBO Water Temp Pro V2 data logger (Onset Company, Bourne, MA) was inserted intravaginally in each cow to record temperature at 30 s intervals. Each data logger was attached to a controlled internal drug-releasing device (CIDR, Pfizer Animal Health, New York, NY) that did not contain hormones.

Simulated Wolf Encounter. Immediately after the pre-exposure assessments, cows within the evaluated group were sorted by previous wolf exposure and moved to 2 adjacent drylot pens separated by a fence line (10 WLF and 10 CON cows in each pen). Pens were 55 x 55 feet, located 0.05 miles from the handling facility, and had no feed or water source. After being housed in their respective pens, CON and WLF cows were immediately subjected to the simulated wolf encounter for 20-min. More specifically, wolf urine (Harmon Wolf Urine Scent; Cass Creek, Grawn, MI) was applied to 12 cotton plugs (Feminine care tampons; Rite Aid, Camp Hill, PA), and plugs were attached to the drylot fence line every 35-feet (6 plugs /pen) prior to any experimental procedures on d 2 and 3. After cows were settled within each dry lot pen, wolf howls previously recorded from the wolf packs residing in Wallowa County, OR, were continuously reproduced using a stereo system (S2 Sports MP3 CD/Radio Boombox; Sony Corporation of America, San Diego, CA) located 30 feet from the dry lot pens, whereas cows had no visual contact with the stereo system. Additionally, 3 trained dogs were conducted using a leash by 2 trained technicians outside the drylot perimeter fence. The dogs utilized were 2 adult German Shepherd females (BW = 76 ± 3.3 lbs) to represent adult wolves, and 1 adult Border Collie × Alaskan Malamute female (BW = 49 lbs) to represent a young wolf. The maximum and minimum distances allowed between dogs and cows were 80 and 15 feet, respectively.

Post-exposure assessments. After 20 min of the simulated wolf encounter, WLF and CON cows were commingled and returned to the handling facility for removal of HOBO data loggers, temperament evaluation, and blood collection as in the pre-exposure assessments. However, cows were also subjected to the simulated wolf encounter during processing for post-exposure assessments. While cows were at the dry lot pens, 3 cotton plugs saturated with wolf urine were attached to the walls of the lead chute at 10-feet intervals immediately prior to the squeeze chute, and 1 similar cotton plug was hung inside the squeeze chute (Silencer Chute; Moly Manufacturing, Lorraine, KS). Wolf howls were reproduced throughout the entire processing. Cows were also exposed for 20 s to the same 3 dogs previously used while restrained in the squeeze chute, but before blood collection, HOBO data loggers removal, or temperament evaluation. Dogs were handled via leash by 2 trained technicians in front of the squeeze chute, and remained 15 feet from the restrained cow.

Immediately after the post-exposure assessments, the group was returned to its original pasture, cotton plugs were removed from the handling facility, and the subsequent group was only evaluated after a 30-min interval to prevent residual wolf scent inside the handling facility during the pre-exposure assessment. Further, the wolf howls were not reproduced and dogs were restrained in an enclosed barn during this 30-min interval to prevent unwarranted visual and auditory stimuli prior to the simulated wolf encounter.

Sample analysis

Individual cow temperament was assessed by chute score and exit velocity as previously described by Cooke et al. (2012). Chute score was assessed by a single technician based on a 5-point scale where: 1 = calm with no movement, 2 = restless movements, 3 = frequent movement with vocalization, 4 = constant movement, vocalization, shaking of the chute, and 5 = violent and continuous struggling.

Exit velocity was assessed immediately by determining the speed of the cow exiting the squeeze chute by measuring rate of travel over a 6 feet distance with an infrared sensor (FarmTek Inc., North Wylie, TX). Further, cows were divided in quintiles according to their exit velocity, within CON and WLF cows on d 0 and within group for pre- and post-exposure assessments, and assigned a score from 1 to 5 (exit score; 1 = cows within the slowest quintile; 5 = cows within the fastest quintile). Individual temperament scores were calculated by averaging cow chute score and exit score.

Temperature data from HOBO loggers were processed using the HOBOware Pro software (version 3.3.2; Onset Company). Only data obtained after the end of the pre-exposure assessments (when cows were gathered and moved to the dry lot pens) to the end of the simulated wolf encounter (when cows were commingled to return to the handling facility) were recorded and compiled into 5-min intervals. Hence, cows had 25 min of recorded body temperature; the initial 5 minutes collected prior to the simulated wolf encounter (pre-exposure assessment) and the remaining 20 min collected during the simulated wolf encounter (post-exposure assessments). Blood samples were collected via jugular venipuncture into a commercial blood collection tube (Vacutainer, 10 mL; Becton Dickinson, Franklin Lakes, NJ) with sodium heparin. After collection, blood samples were placed immediately on ice, centrifuged (2,500 × *g* for 30 min; 4°C) for plasma harvest, and stored at -80°C on the same day of collection. A bovine-specific commercial ELISA kit was used to determine plasma concentration of cortisol (Endocrine Technologies Inc., Newark, CA).

Statistical analysis

Pen within the evaluated group was considered the experimental unit. All data were analyzed using the MIXED procedure of SAS (SAS Inst., Inc.; version 9.3) and Satterthwaite approximation to determine the denominator df for the tests of fixed effects. Significance was set at $P \leq 0.05$, and tendencies were determined if $P > 0.05$ and ≤ 0.10.

Results

The main hypothesis of this experiment was that the mere presence of wolf packs near cattle herds affects temperament and stimulates physiological stress responses known to impair cattle productivity and welfare (Cooke et al., 2009; Cooke et al., 2012; Francisco et al., 2012), particularly in herds previously subjected to wolf predation (Creel and Christianson, 2008; Boonstra, 2013). To address this hypothesis, mature beef cows were subjected to an experimental model designed to simulate a wolf encounter, which was based on wolf scent, pre-recorded wolf howls, and 3 canines physically similar to wolves. Accordingly, wolf scent and recorded howls have been successfully used to mimic wolf presence (Moen et al., 1978; Kluever et al., 2009), given that such stimuli can elicit similar behavioral or physiological responses by prey animals compared with the actual presence of the predator (Kats and Dill, 1998; Apfelbach et al., 2005). Likewise, Kluever et al. (2009) suggested that cattle may acquire a generalized fear response to domestic dogs, perhaps due to the physical and stalking predation characteristics shared among all canids (Nowak, 1999).

It is also important to note that WLF and CON cows originated from different herds, and were reared in different management schemes and environments. Hence, the impact of previous wolf exposure on the temperament and stress-related parameters evaluated herein cannot be completely distinguished from cow source. To address this concern, WLF and CON cows were commingled to receive the same management for 50 d prior to the beginning of the experiment, and were processed weekly to familiarize all cows to personnel and handling facilities. But more importantly, the temperament and physiological parameters evaluated herein are not being directly compared between CON and WLF cows. Instead, these parameters are being evaluated within each cow based on the changes between pre- and post-exposure values. Both herds were also occasionally exposed to herding dogs and reared in areas with large populations of other canids such as coyotes and foxes (Idaho Fish and Game, 2013; Oregon Department of Fish and Wildlife, 2013b). Therefore, differences in temperament and physiological responses between WLF and CON cows following the simulated wolf encounter should be mainly attributed to previous exposure to wolves, and not to interactions with canids in general.

Upon the beginning of the simulated wolf encounter, all WLF groups immediately bunched-up in the farthest corner of the drylot pen, and maintained this disposition during the entire process (Figure 1). Conversely, CON cows remained dispersed within the drylot pen (Figure 1). This behavioral difference suggests that cattle previously predated by wolves immediately adopt a fear-related protective behavior after perceiving signs of wolf presence, whereas the same outcome may not be observed in cattle unfamiliar with wolves.

Figure 1. Behavioral responses of beef cows during the simulated wolf encounter.

Chute score increased (*P* = 0.01) from pre- to post-exposure assessment in WLF cows but did not change in CON cows (*P* = 0.72), indicating that the simulated wolf encounter increased fear-induced agitation during chute restraining only in WLF cows (Burrow, 1997). Accordingly, WLF cows had a greater (*P* < 0.01) positive change in chute score from pre- to post-exposure assessment compared with CON cows (Table 1). Exit velocity decreased (*P* = 0.01) from pre- to post-exposure assessment in CON cows, which may be associated with fatigue caused by the experimental procedures, but did not change (*P* = 0.97) in WLF cows. Hence, CON had a greater (*P* = 0.05) negative change in exit velocity from pre- to post-exposure assessment (Table 1), suggesting that fear-related responses to the simulated wolf encounter prevented the fatigue-induced decrease in exit velocity of WLF cows. Given that temperament score is based on chute score and exit velocity, this parameter also increased (*P* = 0.01) from pre- to post-exposure assessment in WLF cows but did not change in CON cows (*P* = 0.75), evidencing that the simulated wolf encounter increased excitability only in WLF cows. Thus, WLF cows had a greater (*P* = 0.01) positive change in temperament score from pre- to post-exposure assessment compared with CON cows (Table 1).

Table 1. Temperament measurements and plasma cortisol concentrations of cows previously exposed (WLF) or not (CON) to wolves, and subjected to a simulated wolf encounter.

Item	WLF	CON	*P*-value
Chute Score (1 to 5)			
Pre-exposure	2.27	1.85	0.01
Post-exposure	3.07	1.81	< 0.01
P-Value [1]	< 0.01	0.72	
Change [2]	0.78	-0.06	< 0.01
Exit Velocity (feet/s)			
Pre-exposure	8.10	5.50	< 0.01
Post-exposure	8.09	4.62	< 0.01
P-Value [1]	0.97	0.01	
Change [2]	-0.01	-0.88	0.05
Temperament Score (1 to 5)			
Pre-exposure	2.97	2.08	< 0.01
Post-exposure	3.37	2.05	< 0.01
P-Value [1]	< 0.01	0.75	
Change [2]	0.40	-0.04	0.01
Plasma cortisol (ng/mL)			
Pre-exposure	17.9	13.1	0.04
Post-exposure	23.7	14.6	< 0.01
P-Value [1]	< 0.01	0.19	
Change [2]	5.8	1.5	<0.01

[1] Time comparison within WLF and CON cows.
[2] Calculated by subtracting pre-exposure values from post-exposure values.

Plasma cortisol concentrations increased (*P* < 0.01) from pre- to post-exposure assessment in WLF cows but did not change (*P* = 0. 19) for CON cows, indicating that the simulated wolf encounter induced a glucocorticoid stress response only in WLF cows (Sapolsky et al., 2000). Accordingly, WLF cows had a greater ($P < 0.01$) positive change in plasma cortisol from pre- to post-exposure assessments compared with CON cows (Table 1). Body temperature increased ($P < 0.01$) for WLF and CON cows during the simulated wolf encounter (Figure 2). This outcome can be attributed to the handling and physical activity that cows endured during the experimental procedures (Mader et al., 2005), in addition to fear-related stress caused by the simulated wolf encounter because increased body temperature is a major component within the neuroendocrine stress response (Charmandari et al., 2005). However, WLF cows had a greater ($P = 0.03$) positive change in body temperature from pre- to post-exposure assessments compared with CON cohorts (0.74 vs. 0.33°F, respectively; SEM = 0.10). Given that WLF and CON cows were handled similarly and walked the same distances during the experimental procedures, this difference detected in body temperature change can be attributed to a greater fear-related stress that WLF cows endured during the simulated wolf encounter.

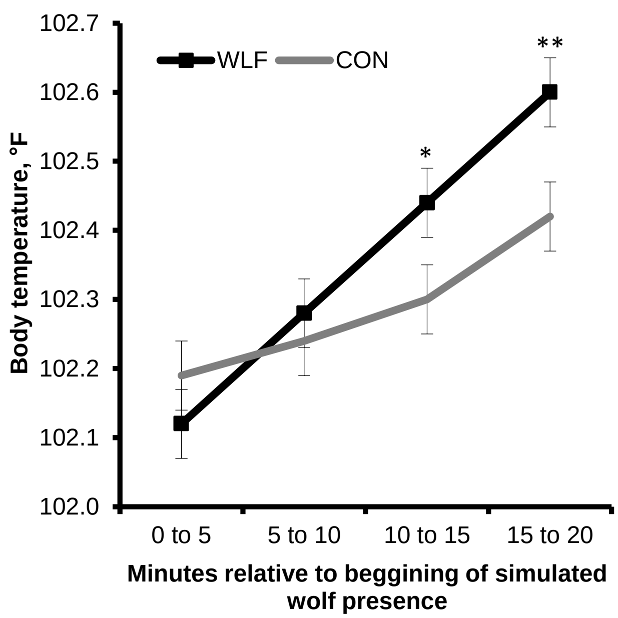

Figure 2. Body temperature of cows previously exposed (WLF) or not (CON) to wolves, and subjected to a simulated wolf encounter. A previous wolf exposure × time interaction was detected (P < 0.01). Treatment comparison within time: ** P = 0.01, * P = 0.05.

Supporting our hypothesis, WLF cows became more excitable and had an increase in plasma cortisol and body temperature following the simulated wolf encounter, suggesting that cows familiar with wolf presence and predation may endure fear-related behavioral and physiological stress responses (Charmandari et al., 2005) when in close proximity with wolves. Conversely, temperament and plasma cortisol concentrations in CON cows were not impacted by the simulated wolf encounter, and the marginal increase in body temperature can be attributed to the handling and physical activity associated with the experimental procedures (Mader et al., 2005). Therefore, wolf presence may not be perceived as a stressor in cows still unfamiliar with predation and interaction with this predator. To our knowledge, no other research has evaluated temperament and physiological stress parameters in beef cows previously exposed or not to wolves, and subjected to a simulated or actual wolf encounter. Hence, results described herein are novel and cannot be properly compared with the limited existing literature within this subject. Nevertheless, Boonstra (2013) described that fear of predation and its behavioral and physiological consequences are based on the anticipatory memory of the attack. Consequently, cows that have not yet been predated by wolves may not experience a fear-related stress response when interacting with wolves for the first time due to the lack of adverse memories from previous predation episodes. In contrast, the behavioral and physiological stress responses detected herein in WLF cows are known to impair performance, reproductive, and health parameters in cattle (Cooke et al., 2009; Cooke et al., 2012; Francisco et al., 2012). These results support the assumption that the impacts of wolf presence and predation on beef cattle systems are not limited to cattle death and injuries, but may also extend to overall productivity and welfare of the herd (Lehmkuhler et al., 2007). Consequently, more research is warranted to directly evaluate the productive and economic consequences that wolves bring to beef cattle operations, including studies with authentic wolf packs, cattle from the same management and genetic background, and assessing cattle performance, reproductive, and health parameters.

Conclusions

Results from this experiment indicate that the simulated wolf encounter increased excitability and fear-related physiological stress responses in cows previously exposed to wolves, but not in cows unfamiliar with this predator. Therefore, the presence of wolf packs near cattle herds may negatively impact beef production systems via predatory activities and subsequent death and injury of animals, as well as by inducing stress responses known to impair cattle productivity and welfare when packs are in close proximity to previously predated herds.

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