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Comparison of natural and artificial odor lures for nilgai (*Boselaphus tragocamelus*) and white-tailed deer (*Odocoileus virginianus*) in South Texas: Developing treatment for cattle fever tick eradication

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A B S T R A C T

Cattle fever ticks (CFT), vectors of bovine babesiosis and anaplasmosis, were eradicated from the United States by 1943, but are frequently reintroduced from neighboring border states of Mexico via stray cattle and wildlife hosts including white-tailed deer (*Odocoileus virginianus*) (WTD) and nilgai antelope (*Boselaphus tragocamelus*). Nilgai antelope are exotic bovids from India that are hosts of CFT, have large home ranges as compared to WTD, thus have the potential to spread CFT through the landscape. Currently, there are no methods to control CFT on nilgai. Odor lures were evaluated to determine if nilgai could be attracted to a central point for development of control methods. Four treatments, nilgai offal a natural odor lure was used as the positive control; and compared to three artificial odors; screw worm lure, volatile fatty acids, citronella oil. Studies were conducted on a free-ranging population of nilgai at the East Foundation's Santa Rosa Ranch (Kenedy Co., near Riviera, Texas, USA). Game cameras were used to document visitation to the lures. In the ten randomly placed transects, 110 nilgai and 104 WTD were photographed. Offal had significantly more visits by nilgai (71% of total visits) than screwworm (15%), VFA (11%), and citronella (4%). For WTD, there was no significant difference in visitation at the lure treatments.

1. Introduction

Nilgai antelope (*Boselaphus tragocamelus*) are in the family Bovidae and closely related to cattle *Bos* spp. They were brought to the United States from India as zoo animals before the mid-1920s and released in South Texas around1930 (Leslie and Sharma, 2009). By the early 1970s, they were distributed in nine Texas (TX) counties and in northeastern Mexico (Presnall, 1958; Sheffield et al., 1983; Leslie and Sharma, 2009). Nilgai are managed in Texas as an exotic species with no regulated hunting seasons. However, they have expanded their range in South Texas and in some areas there are more nilgai per ha than native ungulates (Mungall and Sheffield, 1994; Moczygemba et al., 2012). Nilgai have large home ranges with mean year-round ranges for females of 8234 ha (range = 851-31,533) and for males of 6626 ha (range = 733-20,864) (Foley et al., in review). In comparison, white-tailed deer (*Odocoileus virginianus*), another wildlife host of cattle fever ticks (CFTs) in southern TX, have much smaller home ranges of approximately 600 ha (Webb et al., 2008; Hellickson et al., 2008). One of the unique aspects of nilgai biology is their formation of communal latrines. This behavior has persisted on their introduced range and latrine formation has been observed near offal which are entrails and internal organs disposed of after field dressing harvested nilgai. Formation of communal latrines may be influenced by the presence of odors such as offal.

Nilgai and white-tailed deer co-exist with cattle in South Texas and they are competent hosts for the two CFT species *Rhipicephalus*

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(*Boophilus*) *microplus* and *Rhipicephalus* (*B.*) *annulatus*, introduced to Texas (Perez de Leon et al., 2012). CFTs transmit an important tick-borne disease, bovine babesiosis caused by *Babesia* (*B. bovis* and *B. bigemina*). Bovine babesiosis was once endemic in the southern United States and caused severe losses to the cattle industry. However, the disease and its vectors were eradicated from the United States by 1943 by the U.S. Department of Agriculture - Animal and Plant Health Inspection Service (USDA-APHIS), the Texas Animal Health Commission and livestock producers, and the cooperation of landowners under the Cattle Fever Tick Eradication Program. Presently, the widespread prevalence of CFTs in neighboring border states of Mexico poses a significant threat to U.S. cattle producers. To prevent reintroduction, a quarantine "buffer" zone between Texas and Mexico was established (Perez de Leon et al., 2012, 2014; Giles et al., 2014). Challenges to CFT incursion in the permanent quarantine zone along the Rio Grande River are more frequent (Giles et al., 2014) due to the increased prevalence of CFT host species such as nilgai (Cardenas-Canales et al., 2011), white-tailed deer (Kistner and Hayes, 1970), stray cattle (*Bos* spp.),other livestock and from interactions between CFT and exotic weeds along the trans-boundary region with Mexico which form a pathogenic landscape that facilitates the invasion and survival of CFTs (Racelis et al., 2012; Esteve-Gassent et al., 2014). Nilgai move widely throughout this environment, are implicated in the spread of CFT in South Texas, and therefore necessitate the establishment of CFT quarantine areas that regulate the movement of cattle and other host animals (Texas Animal Health Commission, 2016).

Methods for control of CFTs on white-tailed deer have been developed which use feeders capable of dispensing acaricide treated corn (Pound et al., 1996). However, there are no known methods to control CFTs on nilgai, other than culling, which reduces the density of susceptible hosts (Messenger, 2014). The current study investigated the use of potential odor lures to attract nilgai as the first step in designing a treatment program to control CFT on nilgai. To increase the probability of trapping success, scent/odor lures are often used as an attractant (Geary, 1984; Mills et al., 2010; Schlexer, 2008). An odor lure is typically a substance that attracts an animal through its sense of smell and is different from bait as it is not intended to be consumed (Schlexer, 2008). These lures exploit an animal's hunger or curiosity, or stimulate social or territorial responses (Wyshinski, 2001). Lures are typically used to attract target animals into control devices such as traps or pharmaceutical delivery systems. Odor lures have been used to attract river otters (*Lutra canadensis*), Everglades mink (*Mustela vison*) to estimate abundance (Humphrey and Zinn, 1982), to attract coyotes (*Canis latrans*) to traps (Turkowski et al., 1983), to estimate density of grizzly bears (*Ursus arctos horribilis*; Romain-Bondi et al., 2004), and for estimation of species distributions (Moruzzi et al., 2002).

Evaluation of attractiveness and selectivity of potential lures is needed to develop control methods, including acaricide or vaccine delivery systems, for nilgai in the context of vector management of the CFT (Martin and Fagre, 1988; McDaniel et al., 2000; Turkowski et al., 1983). An efficient way to determine lure selectivity is to combine scent lures with remote trail cameras which are triggered by movement to record animal activity (Moruzzi et al., 2002; Campbell and Long, 2008). Trail cameras offer many advantages for wildlife research including definitive species identification, multiple species detection, and a permanent photo record (Schlexer, 2008; Texas A&M Agrilife Extension, 2009). The current study used trail cameras to investigate the effectiveness of potential artificial odor lures in attracting nilgai as compared to offal, which is anecdotally reported to be attractive to nilgai. WTD were included in the study because they were commonly encountered in preliminary tests of the lures and are also important wildlife hosts of CFT in South Texas. This study tested the hypothesis that nilgai are attracted equally to offal and synthetic compounds that mimic offal.

2. Material and methods

2.1. Study site

The study was conducted from August to October 2016 on the East Foundation's Santa Rosa Ranch, a 9000 ha ranch located in Kenedy County, Texas (26°55′N, −97°42′E) (Fig. 1). The ranch runs a cow-calf operation designed to support wildlife conservation and other public benefits of ranching and private land stewardship. Nilgai are common and surveys conducted in 2016 reported a population density of 4.5/km^2 (TC & AO unpublished data) The ranch is situated in a sub-tropical region which receives an average rainfall of 60 cm of rainfall annually with average daily temperatures ranging from 19 to 27 °C. The study site is characterized by a dense chaparral honey mesquite (*Prosopis glandulosa* Torr.) and huisache (*Acacia farnesiana* (L.) Willd.) savannah, and oak woodlands (*Quercus virginiana* Mill.).

2.2. Treatment lures

Four treatment lures were evaluated for their attractiveness to nilgai. The study used nilgai offal, a natural odor lure, as a positive control and compared it with three artificial odor lures. Screwworm lure and volatile fatty acids were chosen because they mimic odors expected to be produced from offal. Citronella oil was included as a strong organic odor that was unrelated to the smell of offal.

2.2.1. Nilgai offal

Ten fresh offals consisting of the internal organs and entrails from the thoracic and abdominal cavities of nilgai harvested on the East Foundation's El Sauz Ranch in Willacy Co., Texas were collected on June 9, 2016. The offals were individually packed in large plastic bags and immediately cooled on ice before transportation to the Cattle Fever Tick Research Laboratory (CFTRL), located in Edinburg, Texas where they were stored at −20 °C until further use. During trials, offals were placed into plastic, 20-L lure buckets. In a pilot study, to extend the effectiveness of nilgai offal, caging of offal was tested by placing offal into an iron mesh cage, but attraction was greater when the offal was placed in uncaged buckets where naturally occurring scavengers such as turkey vultures (*Cathartes aura*) and coyotes (*Canis latrans*) had access. Feeding and defecation by carnivores was considered to be a natural part of the odor lure and more similar to offal observed by hunters to be attractive to nilgai.

2.2.2. Screw worm lure

This lure was developed to mimic rotting flesh to attract the livestock pest, screwworm fly *Cochliomyia hominivorax* (Coquerel). It was selected for testing because of its strong odor and similarity to the smell of rotting offal. The screwworm lure was prepared as per the method of Mackley and Brown (1984) with slight modifications. Briefly, butanol, iso-butanol, and acetic acid (each 187 ml) were mixed with butyric acid and pentanoic acid (62 ml each). Phenol (50 g), p-cresol (50 g), benzoic acid (12 g) and indole (12 g) were added and mixed properly. Finally, dimethyl disulfide (187 ml) was added and the lure was then aliquoted in 50 ml graduated tubes and stored at room temperature until further use. All chemicals for the lure were purchased from Sigma-Aldrich, USA and formulated at the CFTRL, prior to deployment in the field.

2.2.3. Volatile fatty acid (VFA) lure

VFA was selected as a treatment because it mimics the smell of

Fig. 1. The location of the study site (Santa Rosa Ranch) near Riviera, TX.

entrails, which are part of offal and has a strong odor. The VFAs in the lure approximated the VFAs in the rumen of a bovine heifer and were prepared using methods described by Dr. Paul Reimer (USDA-ARS, Madison, WI). The lure mixture contained 100 mM acetic acid, 24 mM propionic acid, 14 mM butyric acid, 1.7 mM valeric acid, 0.2 mM caproic acid, 1.7 mM iso-butyric acid, 1.2 mM 2-methyl-butyric acid and 1.2 mM iso-valeric acid. Lure was formulated at the CFTRL, and stored in an amber glass bottle at room temperature prior to deployment in the field.

2.2.4. Citronella oil

Citronella oil was selected because it produces a pungent and long-lasting odor, especially under higher temperatures. It does not mimic offal, so this treatment tests the hypothesis that nilgai are generally attracted to strong odors other than offal. It is derived from *Cymbopogon* spp., (lemongrass), which is a native to India throughout the range of nilgai. The oil was purchased from Sigma-Aldrich, USA and stored at the CFTRL, prior to deployment in the field.

2.3. Lure sites

Four treatments of nilgai offal (control), screw worm lure, VFA, and citronella oil were established on each of ten transects at the East Foundation's Santa Rosa Ranch (Kenedy Co., TX). Random points $\geq$1 km apart were established within the study site and used as the beginning of transects oriented NE to SW to be perpendicular to the prevailing SE wind to avoid mixing of the odor plumes. Each transect was marked at four points at 0, 100, 200, and 300 m to deploy the four lures (Fig. 2).

2.4. Preparation of transects

At each site in each transect a plastic 20 L bucket was recessed in hole (0.5 m deep) so that the vent openings were level with the soil surface (at the conclusion of study, buckets were removed for disposal) (Fig. 3). Four Moultrie A-5 Trail Cameras (Gen2; Model: MCG-12688) with motion sensors and 12-LED near-infrared flash with a 15 m range to get clear pictures even at night were mounted on wooden stakes at approximately 1 m height around the bucket on each site. Cameras were placed strategically within a 15 m radius of the lure, keeping in view the direction of wind flow and existing animal trails to cover all areas. Vegetation in front of the cameras was removed to reduce the occurrence of vegetation triggering the camera's motion detector. Cameras were set to take three pictures per triggering event to capture the animal's direction of travel or identify unique individuals. The images were stored on 16 GB digital (SD) cards.

Lure assignments for each transect were selected at random. At the offal site the partially thawed offal was placed over the bucket and allowed to settle into the bucket. At the artificial lure sites, 100 ml of lure were poured into the buckets. For screwworm lure sites, an additional screw cap vial fitted with a dental wick (Richmond Dental & Medical, Charlotte, NC) and 50 ml of lure was added to the bucket. The vial with dental wick was developed to extend the life of the screwworm lure given its high rate of volatility in warm weather, which was not an issue with the other lures, due to their composition. Immediately after the lures were deployed, the game cameras were activated. Memory cards of all cameras were replaced after two weeks in order to continue data collection. The study was carried out in two phases starting Aug. 8-Sept. 6 and Sept. 9-Oct. 14, 2016 with 5 transects deployed during each period.

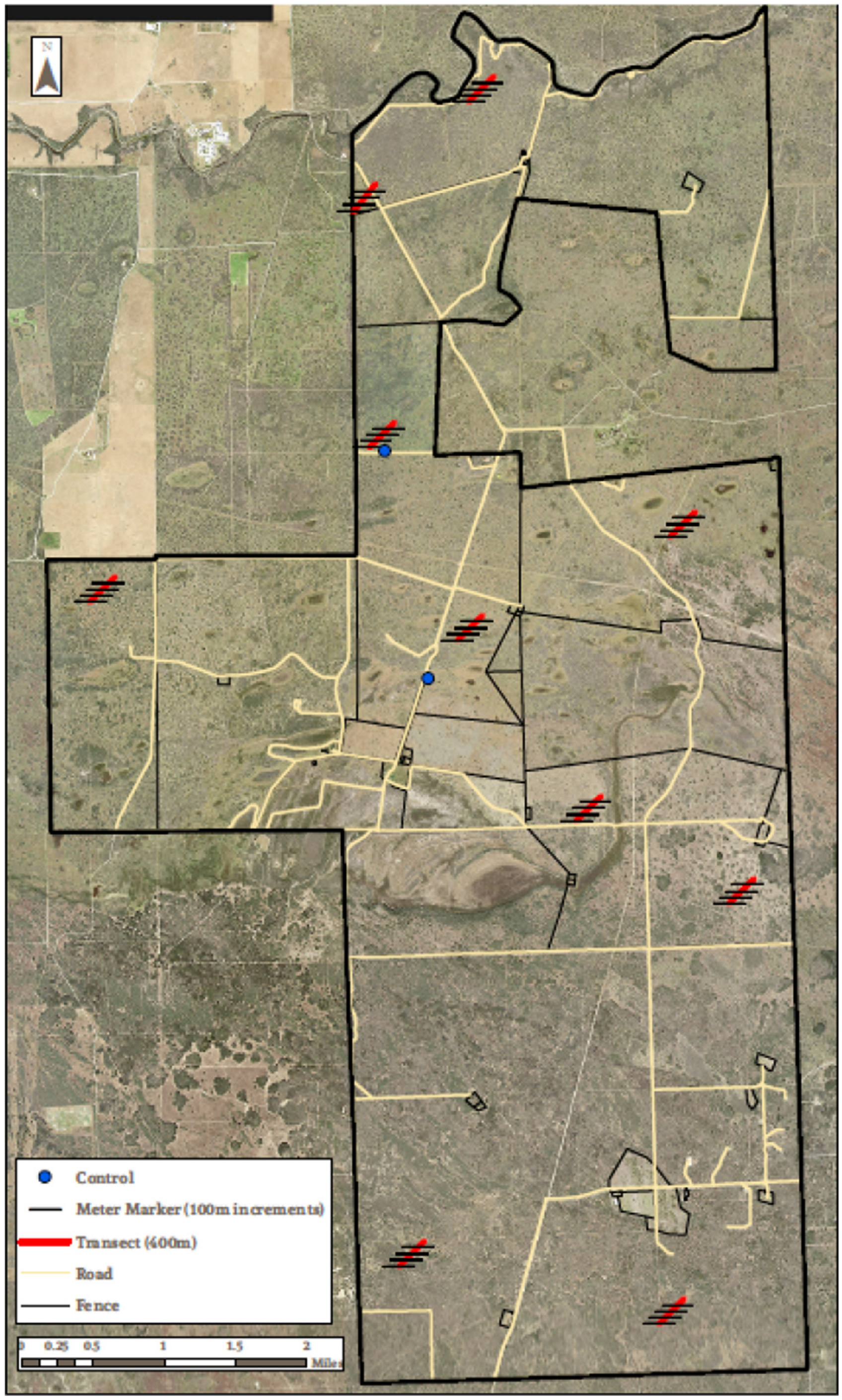

Fig. 2. Locations of nilgai lure transects (red bars) at the Santa Rosa Ranch near Riviera, TX. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2.5. Data collection and analysis

Attraction to lures was recorded by visitation to sites determined by examination of digital images. We use the term animals to refer to both nilgai and WTD. The number of visits by animals to each lure was tabulated by species and transect. We defined a visit as a clearly identifiable picture of a nilgai or WTD. In some cases there were multiple pictures of the same animal during a short period of time. In these cases we counted this as one visitation. We modeled separately the number of nilgai and WTD visits to each lure assuming a negative binomial distribution with a log link function in the GLIMMIX procedure of SAS 9.3 (SAS, 2012). Lure was a fixed effect and transect a random effect in our analysis. Standard errors were presented with all means.

3. Results

A total of 214 target animal visits were captured at all lures of the ten transects consisting of 110 nilgai and 104 WTD. Additionally, cattle (*Bos taurus* X *indicus*) were documented visiting some of

Fig. 3. Lure bucket recessed into soil at each treatment location at the Santa Rosa Ranch.

the transects. Other non-target animas including coyotes, raccoons (*Procyon lotor*) opossums (*Didelphis virginiana*) feral pigs (*Sus scrofa*) and turkey vultures (*Cathartes aura*) were observed infrequently only at the offal sites.

3.1. Transect wise nilgai and WTD visits

Among the ten transects deployed for the study, the number of visits of animals varied ($P > 0.05$) among the various transects over the course of the study. Number of visits of all animals averaged 21.4 ± 5.48 per transect (range = 3–57), whereas nilgai averaged 11.0 ± 4.27 visits/transect (range 1–46) and WTD averaged 10.4 ± 3.13 visits/transect range (0–30).

3.2. Lure wise nilgai and WTD visits

The offal lure used as a positive control received 102 total visits (78 nilgai, 24 WTD) followed by screwworm lure with 48 visits (16 nilgai, 32 WTD). VFA and citronella oil lures received 32 visits each (Table 1, Fig. 4A). The offal lure attracted 48% of total animals and 71% of nilgai attracted to all lures during the study and was significantly greater ($P < 0.05$) than the other lures for nilgai (Fig. 4B). The VFA lure only attracted 11% of the total nilgai. Similarly, citronella oil accounted for only 4% of nilgai visits. There was no significant difference in visitation of lure sites between the screwworm and VFA (Table 1). However we only documented nilgai defecating at the offal and screwworm lure sites (Fig. 5). For WTD, The highest percent (31%) of visits were to the screwworm lure although there was no significant difference between treatments (Fig. 4C).

4. Discussion

After their initial release in the 1930s, the population and distribution of nilgai in South Texas have increased despite year-round hunting without regulations. Nilgai habitat in South Texas grasslands is similar to their native range in India. In India, nilgai generally avoid dense forest and prefer level or undulating plains covered with grass and patches of scrubs (Sankar et al., 2004) which is characteristic of the South Texas plains. In India, there are also several large predators including tigers, (*Panthera tigris*); lions, (*Panthera leo*); leopards, (*Panthera pardus*); wolves, (*Canis lupus*); and striped hyenas, (*Hyaena hyaena*); that effect their survival, reproduction and behavior. In Texas, nilgai distribution is likely to increase over time given the favorable local conditions and relocation of animals for hunting purposes. This may further complicate the negative interactions nilgai have with cattle production and overall success of CFT eradication. Further, a recent research study from South Texas revealed wide-ranging movements in nilgai with mean year-round home range sizes were similar between sexes but highly variable between females (8234 ha, range = 851-31,533) and males (6626 ha, range = 733-20,864) (Foley et al., In Review). This underscores associated risks of nilgai moving CFTs large distances throughout the Temporary Cattle Fever Tick Preventative Quarantine Areas in eastern Cameron and Willacy Counties in Texas and to the north (Texas Animal Health Commission, 2016). Therefore, developing a lure and delivery system for CFT control measures on nilgai is critical to the eradication program to prevent spread of this livestock disease vector in South Texas and beyond. Further, data on WTD attraction towards the employed lures were included as they also serve as an alternate host of CFT (Kistner and Hayes, 1970) and are involved in its spread.

Even though, the offal lure attracted significantly more nilgai in our study, the screwworm lure was the only other lure to induce defecation and possible latrine formation. Therefore the screwworm lure was functionally more useful than the other artificial lures. It is possible that higher doses of screw worm lure could improve attraction to a level more similar to offal. The screwworm lure in buckets has been informally tested by USDA Wildlife Services and it improved the efficiency of ground harvesting of nilgai (David Trevino, personal communication). Future tests should focus on higher rates of the screwworm lure and use the numbers of nilgai visitations at offal sites as a benchmark for a successful lure. Use of offal is logistically difficult and may not be feasible for wide spread deployment as a lure for CFT control on nilgai.

Other natural lures including extracts of plant compounds from species in the native range of nilgai in India should be investigated. Nilgai are known to be attracted to the fresh flowers of the mahua tree (*Madhuca longifolia*) in India (NS, personal communication). Although this plant species does not occur in the U.S., the volatile odor compound, jasmonic acid has been isolated from this plant and should be investigated as a lure. Other nilgai attractants may be known from traditional observation in India and could be gathered through written and oral surveys. These surveys could also reveal compounds or plant materials that could be used as baits. These baits could be medicated with acaricides for CFT control in a similar

Table 1
Lure wise visits of animals on all transect at the East Foundation's Santa Rosa Ranch, near Riviera, TX.

Lure	Total Animal No. (Mean ± SE)	Nilgai No. (Mean ± SE)	WTD No. (Mean ± SE)
Offal (control)	102 (10.2 ± 4.38)[a]	78 (7.8 ± 3.87)[a]	24 (2.4 ± 1.18)[a]
SW	48 (4.8 ± 1.76)[b]	16 (1.6 ± 0.42)[b]	32 (3.2 ± 1.68)[a]
VFA	32 (3.2 ± 1.25)[b]	12 (1.2 ± 0.51)[b]	20 (2.0 ± 1.11)[a]
Citronella	32 (3.2 ± 1.20)[b]	4 (0.4 ± 0.16)[c]	28 (2.8 ± 1.18)[a]

*Letters in each column represent a significant difference.

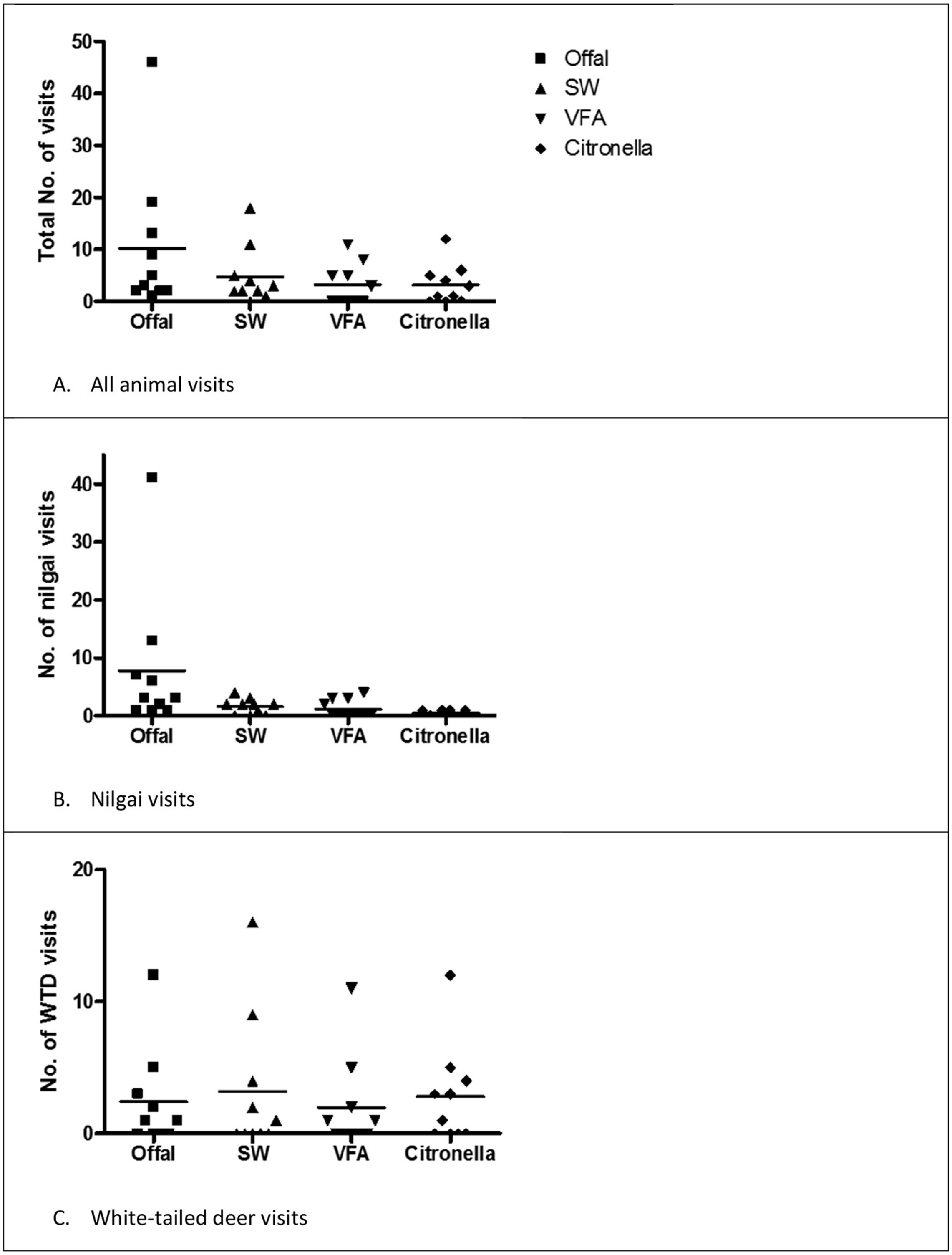

Fig. 4. Distribution of animal visits to lure sites at the Santa Rosa Ranch, near Riviera, TX.

way to corn feeders, which have been utilized in the past for CFT control on WTD (Pound et al., 1996). Future studies should investigate both lures and baits as potential control options for CFT on nilgai.

This study focused on the attraction of nilgai to a central location where they could be potentially be treated for CFT control. It is possible that an effective acaricide or vaccine delivery system could be used in the vicinity directly existing latrines where the local nilgai population is already gathering. More research is needed on nilgai latrine ecology to determine if they could function as a 'lure site' for treatment of CFTs. It may also be possible to combine lures with latrines to get maximum attraction of nilgai in a local area.

Considering the serious challenges of controlling CFTs on nilgai, development of methods to use artificial lures and existing latrines

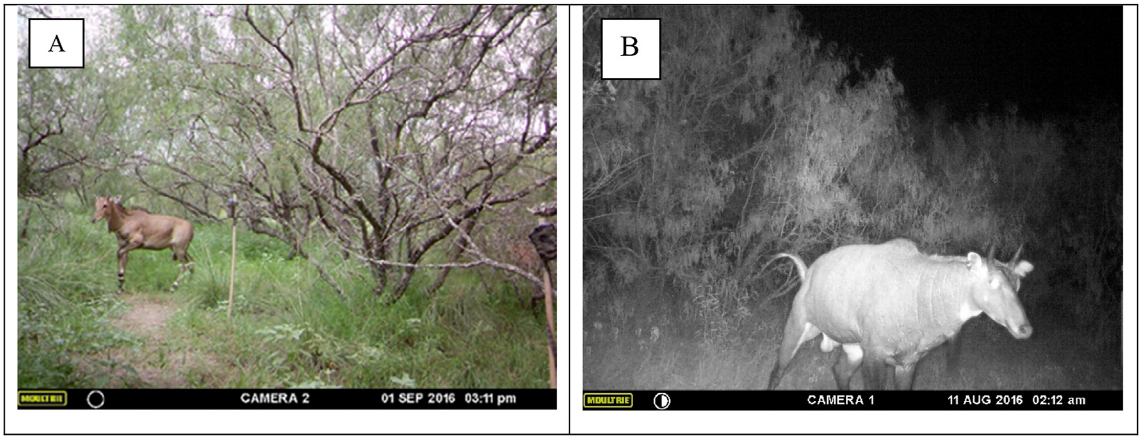

Fig. 5. Nilgai cow visiting lure site (A) and (B) nilgai bull defecating at offal lure site at the East Foundation's Santa Rosa Ranch, near Riviera, TX.

as lures is critically needed towards designing a delivery system for acaricides/vaccines for these animals in their natural habitat. A lure and delivery system would eliminate the present hurdle of capturing and restraining of these animals for the administration of these acaricides/vaccines. Future studies are also needed to improve the attractiveness of artificial lures which mimic offal or specific food items that are known to be attractive to nilgai. Ultimately, a strategy to effectively attract and control the CFT on nilgai could potentially replace aerial and ground harvesting efforts to safeguard Texas and U.S. cattle.

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