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DiazaCon reduces black-tailed prairie dog reproduction in Colorado

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Abstract

Context. Black-tailed prairie dogs (*Cynomys ludovicianus*) often come into conflict with humans in urban natural areas adjacent to private property by destroying plants and denuding the landscape. There is a diversity of attitudes related to urban prairie dog management in Colorado and interest in non-lethal methods is increasing. Therefore, the development of non-lethal methods that allow the existence of prairie dogs and mitigate damage related to population density is needed.

Aims. The efficacy of DiazaCon (20,25-diazacholesterol dihydrochloride, a cholesterol analogue) as an oral contraceptive bait for reducing reproduction in black-tailed prairie dogs was tested. Persistence of DiazaCon in black-tailed prairie dogs 11 months after initial application was also determined.

Methods. Prairie dogs were baited with DiazaCon-coated rolled oats or control bait for 10 days over a 20-day period in November 2007 on one control and one treatment site. Sites were randomly assigned to a treatment group. Visual counts were used 8 months post-treatment (June/July 2008) to determine pup : adult ratios on both sites. Prairie dogs on both sites were trapped 11 months post-treatment (September/October 2008) to gather mass and age data to assess juvenile : adult ratios and to collect blood samples for determination of serum cholesterol and desmosterol concentrations.

Key results. DiazaCon treatment reduced the number of pups per adult by 95.5% when compared with the control site in June/July 2008 (0.09 ± 0.02 , $n = 10$, number of observation days) versus 2.0 ± 0.2 ($n = 10$). Free serum cholesterol levels in treated animals ($370.7 \mu\text{g mL}^{-1}$) were lower than control animal levels ($411.6 \mu\text{g mL}^{-1}$, $P < 0.015$), while treated desmosterol levels ($14.5 \mu\text{g mL}^{-1}$) were higher than controls ($0.35 \mu\text{g mL}^{-1}$, $P < 0.02$) 11 months after ingestion of DiazaCon bait. However, these differences would likely not have been sufficient to prevent successful breeding in the upcoming breeding season.

Conclusions. DiazaCon is an effective contraceptive in black-tailed prairie dogs. It is probably reversible and will likely affect breeding success for only one breeding season. DiazaCon may provide an additional tool to help manage urban populations of prairie dogs in locations where lethal control is unacceptable.

Implications. The need for further research into secondary hazards to non-target animals, such as predators, is discussed. Recommendations for field application are also discussed.

Additional keywords: contraception, *Cynomys ludovicianus*, population reduction, reproduction, 20,25-diazacholesterol dihydrochloride.

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Introduction

Black-tailed prairie dogs (*Cynomys ludovicianus*) are one of five species of prairie dogs found in North America. Their distribution covers the Great Plains from northern Mexico to southern Canada. Although they currently occupy less than 2% of their original range (Miller *et al.* 1990, 1994, 2000), they are frequently the subject of controversy. Prairie dogs are considered a keystone species in the mixed-grass ecosystem, having numerous positive interactions with other plant and animal species (Kotliar *et al.* 1999). Their burrows aerate the soil and provide shelter for other wildlife, and they serve as prey for a wide variety of predators (Graves 2001). Human attitudes towards prairie dogs vary widely, from great appreciation and interest in urban residents

to a more antagonistic, low-concern view in rural, largely livestock-dependent areas (Lybecker *et al.* 2002).

Because of the limited opportunities for dispersal in urban environments, prairie dogs can exist there in densities up to five times higher than in rural environments, which can lead to conflicts with humans (Johnson and Collinge 2004; Magle *et al.* 2007). In urban and suburban environments, conflicts stem from degradation of habitats through burrowing and grazing, damage to ornamental vegetation, and the spread of colonies into human neighbourhoods (Hygnstrom and Virchow 1994; Zinn and Andelt 1999; Witmer *et al.* 2000).

Prairie dog habitat along the Front Range in Colorado is highly fragmented and many urban colonies exist in small natural areas

(Magle and Crooks 2009). Some of these areas are officially designated as natural areas by municipalities, and others exist in highway rights-of-way or vacant lots. Management of urban and suburban prairie dogs is highly controversial, and municipalities are charged with maintaining colonies while minimising damage associated with overgrazing and colony expansion (Witmer *et al.* 2000).

In 1991, prairie dog populations in Fort Collins, Colorado occupied ~836 ha, 82% of which occurred in designated natural areas (Fort Collins Natural Resources Division 1998). A survey of Fort Collins residents in 1993 showed that residents who experienced no prairie dog-related damage supported relocation over lethal control. Residents who experienced conflicts with prairie dogs were more likely to support lethal control measures (Zinn and Andelt 1999). Both groups of stakeholders were willing to support a combination of control and preservation, rating protection of property and the presence of raptors as important outcomes of management. Because of the diverse attitudes related to prairie dog management, non-lethal methods that allow the existence of prairie dogs but help reduce damage related to population growth are needed.

Management of prairie dogs has included lethal control such as poisoning and fumigation, and non-lethal measures such as barriers and relocation (Franklin and Garrett 1989; Robinette *et al.* 1995; Andelt and Hopper 1998). Poisoning, while potentially effective, is frequently restricted or regulated and may pose hazards to pets, children and non-target species. The use of toxicants and fumigants can be very controversial, especially in urban-suburban settings, and is prohibited in many contexts (Witmer *et al.* 2000). Barriers and relocation tend to be expensive, are not always effective, are dependent on available sites and, in the case of some relocations, can be highly detrimental to both transplants and conspecifics at the transplant site (Franklin and Garrett 1989; Hygnstrom 1996; Truett *et al.* 2001; Witmer *et al.* 2008; Massei *et al.* 2010). The cost of control can outweigh the benefits (Collins *et al.* 1984). Survival and reproductive rates increase after a population declines, whether caused by human-initiated lethal measures or by natural factors such as plague outbreaks (Knowles 1986; Cully 1997).

Contraception is a non-lethal management technique that could help reduce the growth of prairie dog colonies. One promising oral contraceptive agent, DiazaCon (20,25-diazacholesterol dihydrochloride), prevents the conversion of desmosterol to cholesterol by inhibiting the Δ_{24} -reductase enzyme (Emmons *et al.* 1982; Yoder *et al.* 2004). Cholesterol is essential for reproduction because it is needed for the production of pregnenolone, the precursor hormone to progesterone, oestradiol and testosterone. Progesterone and oestradiol are needed for ovarian follicular development and ovulation, and testosterone is necessary for sperm production. Reducing cholesterol reduces reproductive steroid hormone synthesis, thereby decreasing reproduction (Yoder *et al.* 2004). Concomitant with decreased cholesterol is an increase in desmosterol, which can be used as a biomarker of DiazaCon consumption (Johnston *et al.* 2003).

Yoder *et al.* (2011) gavaged grey squirrels (*Sciurus carolinensis*) for two days with aqueous DiazaCon solutions (either 50 or 100 mg mL⁻¹), followed by feeding them

DiazaCon-coated peanuts for 8 days with target doses of either 50 or 100 mg kg⁻¹. Serum cholesterol was significantly reduced by $\geq 40\%$ when compared with controls for 2 months following administration in both treatment groups, indicating that squirrels would likely have been contracepted. Nash *et al.* (2006) reported prairie dogs treated with 30–45 mg DiazaCon per kg body mass for 10 days spread out over a 3-week period exhibited a 41% decrease in plasma cholesterol concentrations and a 47% decrease in reproduction (juveniles/adult). Treatment of prairie dogs began immediately before the breeding season, which runs from February to March. It is likely that earlier treatment with DiazaCon would have had a greater inhibitory effect (Nash *et al.* 2006). Therefore, we conducted a study that implemented an earlier treatment date.

This study had three objectives, to determine: (1) the efficacy of DiazaCon for the reproductive control of black-tailed prairie dogs; (2) the effect on contraceptive efficacy of starting treatment before the breeding season; and (3) whether the effects of DiazaCon administration persist in animals 11 months after treatment.

Materials and methods

The experimental protocol (QA-1388) describing this study was reviewed and approved by the National Wildlife Research Center's Animal Care and Use Committee and complied with the Animal Welfare Act.

Study area

The study area was located ~4.5 km south-west of Fort Collins, Colorado at a natural area maintained by the City of Fort Collins. The area encompassed ~452 ha of shortgrass prairie and foothills shrubland with several kilometres of hiking trails available to the public. Off-trail access by the public was prohibited. Both control and treatment sites were located at this area. A customized SAS statistical program was used to randomly assign the two sites to either the treatment or control group (SAS Institute Inc. 2003).

The control site was 3.3 ha and the treatment site was 4.1 ha (Fig. 1) and the two sites were 113.1 m apart. Within both control and treatment sites, two 1-ha plots (named north and south) were delineated using stakes with fluorescent orange or pink flagging to make a total of four plots.

Bait manufacture and site baiting

Batches of treatment bait were manufactured using rolled oats, molasses (3.5% w w⁻¹) and DiazaCon (0.22% w w⁻¹). Control bait was produced in the same manner, omitting DiazaCon. A 50 g subsample of each batch of bait was removed and stored at -70°C until analysed for DiazaCon content. The bait was either used the day it was manufactured or stored for < 2 weeks at 4°C until needed.

Prairie dogs were pre-baited with control bait for 6 days over an 8-day period in early November 2007. Approximately 22.5 g bait (~0.25 cup) was placed on the burrow mound just outside the entrance of all active burrows. Since a single prairie dog can maintain up to five burrows (Hoogland 1995), baiting all active burrows was necessary to assure complete exposure to the bait. At the end of the pre-baiting period, treatment began

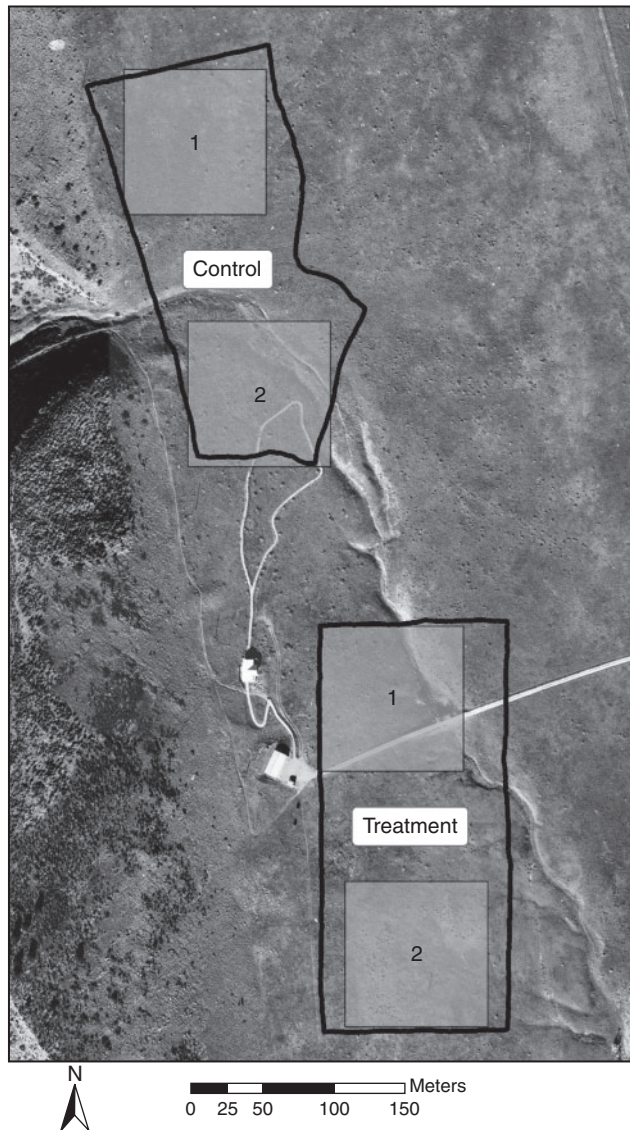


Fig. 1. Aerial map of experimental site showing control and treatment plots.

and ~22.5 g of either control or treated bait was placed on the active mounds. No baiting occurred on days when prairie dogs were unlikely to come above ground (e.g. raining, snowing, temperature < 0°C). Baiting occurred on 10 days over a 20-day period. The target dose was 50 mg DiazaCon kg⁻¹ body mass day⁻¹ for each animal and was based on an average body mass of 1 kg and consumption of ~22.5 g of bait day⁻¹.

Visual obstruction assessment

Visual counting began in mid-June 2008 when pups (juveniles recently emerged from the burrows) were easily visible. An estimate of visual obstruction (the percentage of each colony that could not be seen from the counting position; Menkens *et al.* 1990) was obtained and used in adjusting visual pup counts for each observer as described below for both the treatment and control sites. A known number of 1 L plastic bottles were painted light brown and placed in both systematic

and random patterns throughout each site. Random placement entailed placing bottles on prairie dog mounds, near clusters of mounds and in a random (not equally spaced) distribution across the plot. Systematic placement was defined as placing bottles on linear transects with prairie dog mounds somewhat influencing the location of transect lines. Transect lines were parallel to each other and placed in a straight line such that the maximum number of prairie dog burrows would be close to each line. A grid system was not used for the placement of transect lines because newly emerged pups were most likely to stay close to their burrows. Observers were not present for the placement of the bottles.

Using binoculars, two observers counted bottles at each site three times (Severson and Plumb 1998) for each pattern of bottle placement (e.g. three times for random placement and three times for systematic placement). The counts were averaged for each site and visual obstruction was estimated using the following equation:

$$\text{Visual obstruction} = 1 - \frac{(\text{bottles counted})}{(\text{actual number of bottles})}$$

Visual obstruction was determined separately for the control and treated sites.

Visual prairie dog counts

Visual prairie dog counts were adjusted for visual obstruction using the following equation (Menkens *et al.* 1990):

$$\text{adjusted count} = \frac{(\text{actual count})}{(1 - (\text{visual obstruction}/100))}$$

Counts of pups and adults were conducted using binoculars on five consecutive weekdays in June and July 2008. All counts occurred on sunny or slightly overcast days with no precipitation. Counts were performed from ≥30 m outside the boundaries of the treatment and control plots to reduce disturbances.

Arrival of personnel at the study site between 0830 and 0900 hours invariably resulted in animals seeking refuge in their burrows, so counting did not commence until prairie dog activity resumed (~30 min). To count the animals, plots were scanned starting at one end of the plot and proceeding to the other end. The total number of animals seen and the number of adults, pups, or unknown-aged prairie dogs was recorded. To avoid double counting, observers did not go back over any section during a count. Observers then waited 15 min before beginning the next count. Family group (coterie) relationships were not assessed.

Prairie dogs on each 1-ha plot were counted four times per day. One person counted both plots on the control site and another person counted both plots on the treated site. Observers alternated between sites each day to reduce observer bias. Every count was started on the control site at the south plot, and every count on the treated site at the north plot. This minimised disturbance because alternating the starting plot each day would have required the observer to walk across one plot to reach the other, creating unnecessary disturbance. Counts were adjusted from each time period as described above.

Table 3. Mean (\pm s.e.m.) serum cholesterol and desmosterol concentrations ($\mu\text{g mL}^{-1}$) of black-tailed prairie dogs (*Cynomys ludovicianus*) trapped during September and October 2008 on control and treated sites in Fort Collins, CO

ND = not detected

			Cholesterol ($\mu\text{g mL}^{-1}$)			Desmosterol ($\mu\text{g mL}^{-1}$)		
			Mean	s.e.m.	<i>n</i>	Mean	s.e.m.	<i>n</i>
Control	Juv	F	403.9	33.1	9	ND	ND	9
	Juv	M	407.2	15.6	6	ND	ND	6
	Ad	F	428.4	20.9	5	ND	ND	5
	Ad	M	412.7	28.8	11	1.0	1.0	11
Treated	Juv	F	280.0	37.0	2	ND	ND	2
	Juv	M	344.0		1	ND		1
	Ad	F	393.3	11.8	13	11.3	3.4	13
	Ad	M	365.3	22.6	16	19.9	8.8	16

concentration in control animals ($0.35 \mu\text{g mL}^{-1}$) was substantially lower than in treated animals ($14.5 \mu\text{g mL}^{-1}$).

Discussion

The ratio of pups per adult (0.09 versus 2.0) was 95.5% lower on the DiazaCon-treated site compared with the control site. This is much greater than the 47% difference achieved by Nash *et al.* (2006). In black-tailed prairie dogs, testosterone peaks around the end of December (Knowles 1987; Hoogland 1995). Nash *et al.* (2006) did not bait until February, after testosterone had peaked in males. Sperm production was probably not impaired in these males, and females likely had already undergone ovarian follicular development by the time DiazaCon took effect. In the current study, DiazaCon was applied a month before peak testosterone levels in males, so spermatogenesis was probably inhibited. Similarly, females most likely did not undergo ovarian follicular development. Earlier treatment probably resulted in higher efficacy.

The ratio of juveniles per adult (0.103 versus 1.07) was 90.4% lower on the treated site than on the control site, somewhat lower than the efficacy indicated by the ratio of pups to adults 3 months earlier (0.09 versus 2.0). This could be due to the difficulty of assigning borderline animals to an age group using body mass. When prairie dogs weighed close to the cutoff weight between juveniles and adults, it was difficult to assign them to an age class. Post-parturition females in particular can weigh roughly similar to an older juvenile (Hoogland 1995, 2003). Some animals that were classified as juveniles might have actually been adults.

The number of adults on the control and treated sites was similar when pups first emerged from burrows in June and July, but the number of adults on the treated site was nearly twice that of the control site in September–October 2008, with much of the increase being due to a 3-fold greater number of adult females on the treated plots compared to the control plots.

This difference may have resulted from immigration from untreated areas. However, dispersal is usually male-biased (Hoogland 1995), much more prevalent in yearling males (≥ 8 months but ≤ 20 months after emergence) than adult males (Hoogland 1995) and occurs most commonly in early to mid-June, which would preclude movement by young-of-the-

year in this study. Most females remain in the coterie in which they were born for their entire life (Hoogland 1995). Over time, a coterie's home territory usually remains roughly constant. However, the number of prairie dogs within the coterie can be highly variable so that prairie dog density within a coterie territory can vary substantially (Hoogland 1995). The increase in adult males and females observed in this study over a 2–3 month period is highly unusual and has not been previously reported (J. Hoogland, pers. comm.)

This study involved a relatively small, local colony. A larger-scale study, in which entire populations are treated, treatments are replicated and all animals individually marked from the outset, is warranted, and could eliminate dispersal and immigration as potentially confounding factors. Additionally, the inferences made herein are specific to the two treatment sites and we do not extrapolate our findings to the population or geographic level. The proximity and similarity of the two treatment sites provided reasonable assurance that resulting differences were due to the treatment.

Adult male and female prairie dogs on the treated site weighed significantly more than those on the control site, and there are likely several reasons for this. Treated females may have weighed more because (1) they did not experience the physiological demands of pregnancy and lactation, and (2) they likely had more time to forage because they were not raising a litter. An even greater factor in the observed weight gain in treated prairie dogs may have been a result of the treatment itself. As discussed previously, DiazaCon reduces the production of steroid hormones necessary for successful gonadal function and subsequent reproduction, and the reduction/elimination of these hormones through treatment may result in a temporary condition similar to castration or ovariectomy. Castrated male prairie dogs in Colorado weighed more than control males (Schwartz 2002). Domestic animals that are spayed or neutered tend to gain body mass (Houpt *et al.* 1979; Fettman *et al.* 1997). Hormonal changes might be partially responsible for the increased body mass (Wallen *et al.* 2002; Martin *et al.* 2006). The body mass results are consistent with another prairie dog field study using a different contraceptive agent (Yoder, unpubl. data). An unintended benefit of annual contraception may be that increased body mass of treated animals might correspond to a higher over-winter survival rate, although the current study did not address this question. Sterilisation of ricefield rats (*Rattus argentiventer*), European rabbits (*Oryctolagus cuniculus*) and brushtail possums (*Trichosurus vulpecula*) resulted in increased survival of sterilised females (Twigg *et al.* 2000; Jacob *et al.* 2004; Ramsey 2005; Williams *et al.* 2007). Immunocontraception of wild mares also resulted in increased longevity (Kirkpatrick and Turner 2007).

Serum cholesterol concentrations from blood sampled in the autumn were 10% lower in prairie dogs from the treated site than from the control site. Previous studies using DiazaCon in birds showed cholesterol must be decreased by ~40% to impair reproduction (Yoder *et al.* 2005). Results from studies using mice and rats were equivocal (Nash, unpubl. data), and data are not available to determine how much cholesterol must be reduced to impair reproduction in small mammals. However, the small difference in cholesterol concentrations indicated

that treated animals would likely be able to produce litters the following breeding season.

Chemical analysis showed that the actual percentage of DiazaCon present in bait was lower than the target of 0.22% DiazaCon ($w w^{-1}$). Even so, the dose used was effective, indicating that a lower dose could be used. The target dose was 50 mg DiazaCon kg^{-1} body mass day^{-1} per animal, whereas the actual dose received was ~ 38 mg DiazaCon kg^{-1} body mass day^{-1} per animal. This estimate was based on each animal consuming 22.5 g of bait day^{-1} and an average body mass of 1.0 kg. The possibility of overdosing was probably very small. Hambourger and Martinez (1968) treated adult male Sprague Dawley rats with increasing intragastric DiazaCon doses ranging from 40 to 700 mg kg^{-1} and calculated the LD50 as 470 ± 44 mg kg^{-1} with lethality beginning at 300 mg kg^{-1} . In the current study, this would have required a single prairie dog to consume the entire bait aliquots of eight separate mounds, an unlikely event.

Further studies need to be conducted to reduce risks to non-target animals, such as mice, rabbits, birds and snakes, which might consume the bait. Such studies would determine the lowest effective dose, and whether prairie dogs would eat bait placed in their burrows. The risk to predators consuming DiazaCon-treated prairie dogs should also be assessed. Studies are needed to determine the effect of contraception on the social structure of the colony.

Results from this study indicate that DiazaCon is an effective reproductive inhibitor in prairie dogs on the local sites tested, and has promise as an effective tool for the management of prairie dog populations.

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